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THE SYNDROME OF CONGENITALLY APLASTIC OVARIES WITH SEXUAL INFANTILISM, HIGH URINARY GONADOTROPINS, SHORT STATURE AND OTHER CONGENITAL ABNORMALITIES

TABULAR PRESENTATION OF TWENTY-FIVE PREVIOUSLY UNPUBLISHED CASES*

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IN 1938, Turner (7) reported 10 instances of a new syndrome, confined to females, and characterized by sexual infantilism, short stature, webbing of the neck, and cubitus valgus. This was the first report in the American literature calling attention to the syndrome, which later was found to include congenitally aplastic ovaries. The next contribution to the understanding of this condition appeared in 1942 when Varney, Kenyon, and Koch (8) described 4 short, sexually retarded females, all of whom excreted abnormally large amounts of urinary gonadotropins; indeed their titers equalled those of castrate or postmenopausal women. On the basis of 7

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* Presented in abbreviated form at the 28th Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, June 29, 1946. Since this paper was sent to press, one of the patients (Case 5) was included in an article by one of us (Minnie B. Goldberg) with Maxwell and Smith as co-authors, which appeared in this Journal, Vol. 7, p. 11, January, 1947.

scattered autopsy reports discovered in the foreign literature, they suggested the importance of ovarian aplasia as one of the cardinal characteristics of the syndrome. Later that same year, Albright, Smith, and Fraser (1) submitted 11 patients of their own, particularly stressing the primary ovarian insufficiency and discussing its differentiation from primary pituitary infantilism. Two of their patients had been examined by means of the peritoneoscope and no ovaries were seen. In 1943, Schneider and McCullagh (5) described 5 cases of "Turner's syndrome." In 1944, Shereshevski (6) reported 6 cases, the characteristics of which seemed to justify their inclusion in this group. One of these cases had been published previously in 1925, but even in 1944 the author was unaware of the articles referred to above and made no mention of congenitally absent ovaries or high urinary gonadotropins. A review of the literature with tabular presentation of most of the previously reported cases was published in 1944 by Wilkins and Fleischmann (9). Their article included also a table of 18 cases in which autopsy or exploratory laparotomy revealed absent or rudimentary ovaries. These authors added 5 cases of their own, 4 of them confirmed by biopsy studies.

"Ovarian agenesis" is the name that Wilkins and Fleischmann selected for this syndrome. We have refrained from employing this title, since in our opinion, it attaches undue and unwarranted significance to this feature, and seems to imply that the other characteristics such as short stature and various congenital abnormalities are consequent to the ovarian aplasia. Such an assumption seems to us most unlikely as it did to the above authors. This will be discussed later in this paper.

As presently accepted, the syndrome is characterized by congenitally aplastic or completely absent ovaries, sexual infantilism, high urinary gonadotropins, and usually by short stature. Other occasional findings are webbed neck, cubitus valgus, coarctation of the aorta, and other congenital abnormalities. To date about 60 cases have been published. We are adding 25 patients, the diagnosis in 5 having been proven by exploratory laparotomy or peritonescopy. Of the remainder, 10 patients were found to have an abnormally high urinary gonadotropin (FSH) titer. In the other 9 patients, the diagnosis is presumptive, based on typical clinical characteristics. To conserve space, the customary detailed presentation of each case is omitted, all pertinent data being compressed into tabular form.

DISCUSSION OF DIAGNOSTIC CRITERIA IN TABLE I

These patients came under our observation at ages varying from 8 to 39 years. It is to be noted, however, that the diagnosis of ovarian aplasia was not entertained in some instances until several years after they were first seen.

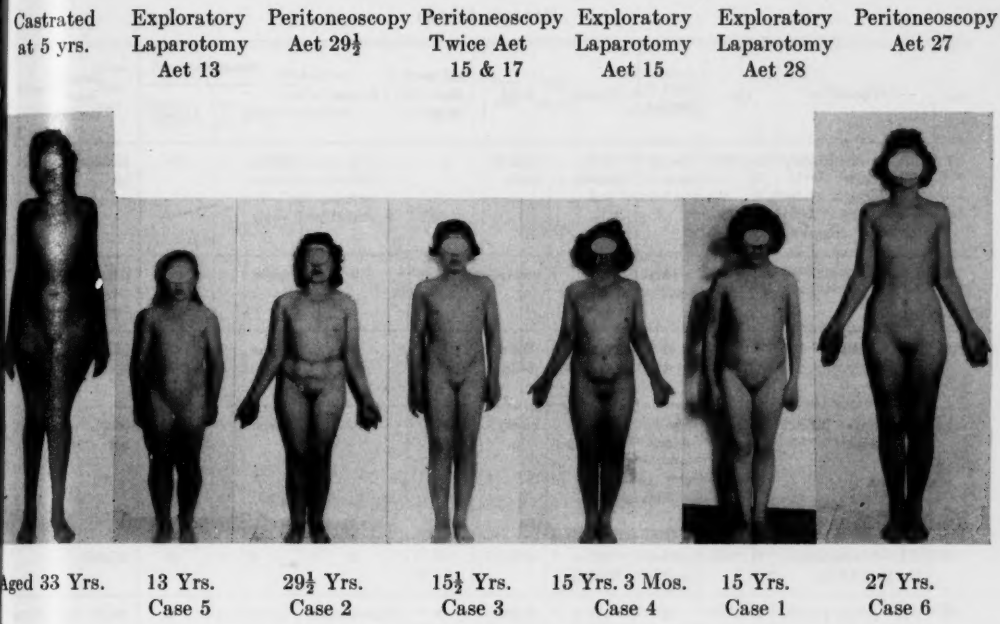

Castrated at 5 yrs.
Exploratory Laparotomy Aet 13
Peritoneoscopy Aet 29½
Peritoneoscopy Twice Aet 15 & 17
Exploratory Laparotomy Aet 15
Exploratory Laparotomy Aet 28
Peritoneoscopy Aet 27
Aged 33 Yrs.
13 Yrs. Case 5
29½ Yrs. Case 2
15½ Yrs. Case 3
15 Yrs. 3 Mos. Case 4
15 Yrs. Case 1
27 Yrs. Case 6

FIG. 1. Proven Ovarian Aplasia

Six cases of ovarian aplasia, 3 proven by exploratory laparotomy and 3 by peritoneoscopy; and for contrast a 33 year old female who was castrated at 5 years of age by two ovariectomies, six months apart, the first because of an ovarian hematoma and the second because of an ovarian cyst with twisted pedicle. She shows the typical disproportionately long extremities and trochanteric fat pads characteristic of so-called preadolescent eunuchism and eunuchoidism. Five of the 6 congenital cases are under 55 inches tall. The sixth is the only exceptionally tall patient in the entire series of 25 cases. See Figure 4 for appearance of pelvis in *case 4*.

It may at once seem surprising that this syndrome should receive consideration in a girl only 13 years of age (the youngest case on record in which the diagnosis has been verified (*case 5*, Figure 1), since the menarche normally often does not occur until 14 to 16 years of age. This girl had been studied for five years previously because of failure to grow. The stunting in association with sexual infantilism aroused the suspicion of ovarian aplasia. Sixteen of 25 patients (64 per cent) were reported as being "always small"; of the remaining 9, the onset of stunting was noted between the ages of 4 and 13. In this group, therefore, short stature was apparent in the majority during the first year of life, and at the latest by the age of 13 years.[1]

[1] In only 1 of our patients (case 8) was there any suggestion of the association of injury with the onset of stunting.

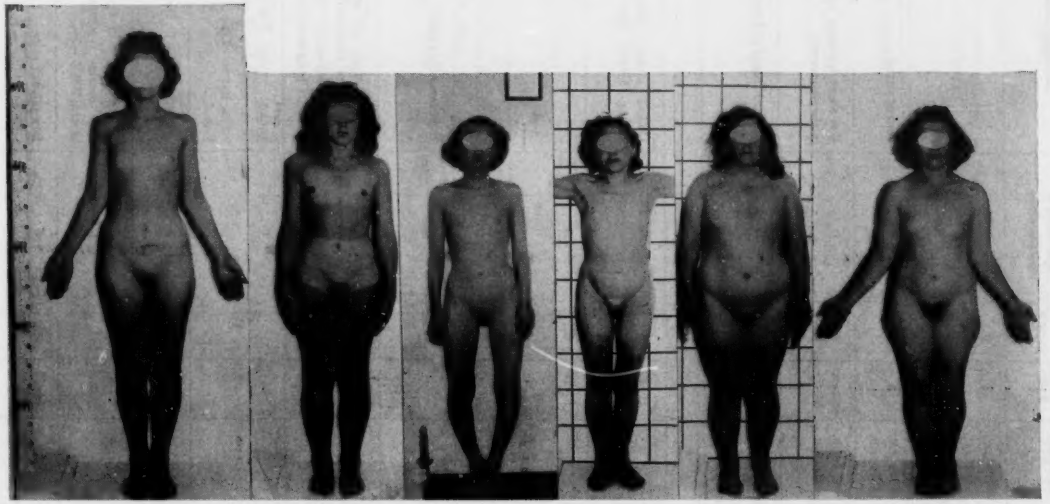
Proven Cases

No	Patient	Age	Onset of Stunting	Height	Build	"Shield-like" Chest	Breasts	Sexual Hair		External, Internal Genitalia	Menstruation
								Pubic	Axillary		
1.	*E.P.*—private patient, Dr. H. Lisser	15 (1927)	Always short	53 in. (134.5 cm.)	Slightly stocky	+	0	Sparse	0	Infantile, ovaries not felt	0
2.	*B.G.*—U-125616	14 (1930)	Always small	47½ in. (120.5 cm.)	Stocky	Slightly+	0	Sparse	Sparse	Infantile, tiny cervix, uterus & ovaries not felt	0
3.	*M.P.*—U-30438	15 (1938)	12	54½ in. (138 cm.)	Slightly stocky	Slight	0	Sparse	0	Infantile, ovaries not felt	0
4.	*M.S.*—private patient, Dr. H. Lisser	15 (1942)	Always short	54½ in. (137.5 cm.)	Stocky	+	0	Sparse	0	Small, ovaries not felt	0
5.	*L.L.*—Private patient, Dr. M. Goldberg	13 (1945)	Always small	49½ in. (126.5 cm.)	Stocky	0	0	0	0	Infantile	0
6.	*P.L.*—private patient, Dr. L. E. Curtis	27 (1946)	0	67½ in. (171 cm.)	Slender	0	Slight development started at 26	Sparse since act. 13	0	Small vagina from plastic operation, ovaries not felt	0
	CASES NOT PROVEN BUT WITH HIGH GONADOTROPINS										
7.	*S.N.*—U1792	21 (1936)	Always small	57 in. (145 cm.)	Thin	0	0	Very sparse	Very sparse	Infantile	0
8.	*D.F.*—private patient, Dr. H. Lisser	15 (1945)	8—after skull fracture	54½ in. (138.5 cm.)	Medium	+	0	Sparse	Sparse	Tiny uterus 1×3 cm. Ovaries not felt	0
9.	*J.L.*—private patient, Dr. L. E Curtis	32 (1945)	Always small	53 in. (134.5 cm.)	Stocky	+	0	Sparse	Sparse	Infantile. Ovaries not felt	0
10.	K.S.—private patient, Dr. M. E. Dailey U-119409	39 (1945)	12	56 in. (142 cm.)	Medium	0	0	Sparse	0	Infantile. Uterus, ovaries not felt	0
11.	*M.A.*—U-118311	27 (1945)	Always small	55½ in. (140 cm.)	Stocky	+	0	Sparse	0	Infantile. Tiny cervix; uterus, ovaries not felt	0
12.	*V.G.*—private patient, Dr. L. E. Curtis	21 (1946)	Always small	54½ in. (138 cm.)	Stocky	+	0	Very sparse	0	Infantile. Ovaries not felt	0

Menstruation	Blood Pressure	Short or Webbed Neck	Cubitus Valgus	Other Congenital Abnormalities	Urinary Gonadotropin Excretion	Bone Age	Osteoporosis	Exploratory Laparotomy or Peritoneoscopy	Additional Data
0	110/70	+		No arterial or venous arcades in mesentery of small intestine (more detail in text)		12–13		Laparotomy, aet 28, on account repeated intestinal hemorrhages. Pelvis empty. No uterus or adnexa	Appeared childlike. Had severe upper bowel hemorrhages. Hgb dropped to 15%. Later repeated hemorrhages caused death. See text under Congenital Abnormalities
0	100/70	+	+		+384 M.U. aet. 29. −480	14–15	+	Peritoneoscopy aet. 29. No uterus, tubes, or ovaries seen	Diagnosed aet. 29. Many raised pigmented moles on face
0	106/64	0	0	0	+80 M.U. ± 144 M.U. −288 M.U.	14–15	0	Peritoneoscopy aet. 15 & 17—no ovaries seen	Estrin in urine, less than 4 gamma
0	90/75	+	+	0	197 M.U.	13–14	0	Lap. aet. 15. Uterus fair sized. No ovary on rt. 2 pin point masses of tissue on left. Strip white tissue on either side	Looks young. Had nephritis or celiac disease aet. 1
0	100/75	0	0	0	+ 27 M.U. − 40 M.U.	11–12	0	Exploratory lap. aet. 13, streak ovaries	Developed keloid in scar postoperative.
0	132/80	0	+	0	+ 96 M.U. −192 M.U. (at aet. 31)	adult	0	Peritoneoscopy aet. 27. No uterus, tubes, or ovaries seen—only a peritoneal fold	Complete gastric achlorhydria. BMR+9%
0	98/60	0	0	Osteogenesis imperfecta tarda	+ 96 M.U. − 192 M.U.	15–16	+	0	Low flat glucose tolerance curve
0	88/50	0	0		+288 M.U. −384 M.U.	13		0	Looks 12 or 13. Considerable birth trauma by forceps. Head asymmetrical since skull fracture at 8. BMR−26%. Cholesterol 278 mg. %. Normal sella. Does not look hypothyroid
0	200/80	+	Slight		+192 M.U. −288 M.U.	Adult	0	0	Gonadotropin value 1 mo. after discontinuing stilbestrol +192 M.U. While on stilbestrol therapy: +9.2 M.U.
0	128/82	0			+288 M.U. −384 M.U.	Adult	0	0	Looked about 50 yrs. old. Married 12 yrs. Occasional pleasure from intercourse. Diabetes mellitus developed aet. 36. Sella slightly deep. Scant head & face hair. Cholesterol 318. BMR+11%
0	135/80	+	+	Anomalous left 4th toe	+165 M.U.	Adult	Slight	0	Many raised pigmented moles on face
0	110/64	+	0	Malformed ears	+384 M.U. −480 M.U.	Adult	0	0	

No.	Patient	Age	Onset of Stunting	Height	Build	"Shield-like" Chest	Breasts	Sexual Hair		External, Internal Genitalia	Menstruation
								Pubic	Axillary		
13.	*K.M.*—private patient, Dr. M. Goldberg	18 (1946)	Always small	59½ in. (150.5 cm.)	Boyish	0	Slight development. Had had previous treatment.	Moderate. Previous treatment.	Moderate	Infantile	0
14.	*C.A.*—U-51073	16 (1946)	Always small	47¾ in. (121 cm.)	Medium	0	0	Very sparse	Very sparse	Small. Uterus & ovaries not felt	0
15.	*H.W.*—U-132055	31 (1946)	Always short	54 in. (137 cm.)	Slightly stocky	Slightly+	Fair development	Normal	Normal	Small cervix & uterus. Ovaries not felt	0
16.	*L.B.*—U-121978	22 (1945)	13	54 in. (137 cm.)	Stocky	0	Well developed pubertal type, small nipples	Sparse	Sparse	Small uterus. Ovaries not felt	One scant period a 17
	PRESUMPTIVE CASES										
17.	*A.C.*—OPD-114588	24 (1924)		56½ in. (143 cm.)	Thin	+	0	Sparse	Sparse	Infantile. Ovaries not felt	0
18.	*M.S.*—U-72366	15 (1929)	Always small	54½ in. (138.5 cm.)	Stocky	+	0	Sparse	0	Infantile	0
19.	*B.H.*—private patient, Dr. H. Lisser	30 (1932)	Always short	58¾ in. (149.5 cm.)	Medium	+	0	Sparse	Sparse	Very small uterus. Ovaries not felt	0
20.	*L.W.*—U-47669	18 (1939)	Under 13	58¼ in. (148 cm.)	Slightly stocky	+	0	Sparse	Sparse	Infantile. Tiny cervix only	0
21.	*H.W.*—U-74112	22 (1941)	4–5	53¾ in. (138 cm.)			0	0	0	Infantile. Ovaries not felt	0
22.	*R.D.*—private patient, Dr. H. Lisser	21 (1942)	Always short	56¾ in. (143 cm.)	Stocky with large legs	+	Underdeveloped	Sparse	Sparse	Infantile, endoscopic shows cervix less than 1 cm. in diameter	0
23.	*D.M.*—private patient, Dr. H. Lisser	15 (1942)	Always short	53¾ in. (136 cm.)	Slightly stocky	+	Underdeveloped	Sparse	0	Infantile. Ovaries not felt	0
24.	*E.H.*—private patient, Dr. H. Lisser	30 (1943)		61½ in. (156 cm.)	Stocky	+	0	Sparse	Moderate	Infantile. Ovaries not felt	0
25.	*K.D.*—private patient, Dr. H. Lisser	16 (1943)	Always short	56¾ in. (143 cm.)	Stocky	+	0	Sparse	0	Ovaries not felt	1 scant p riod 4 mo before

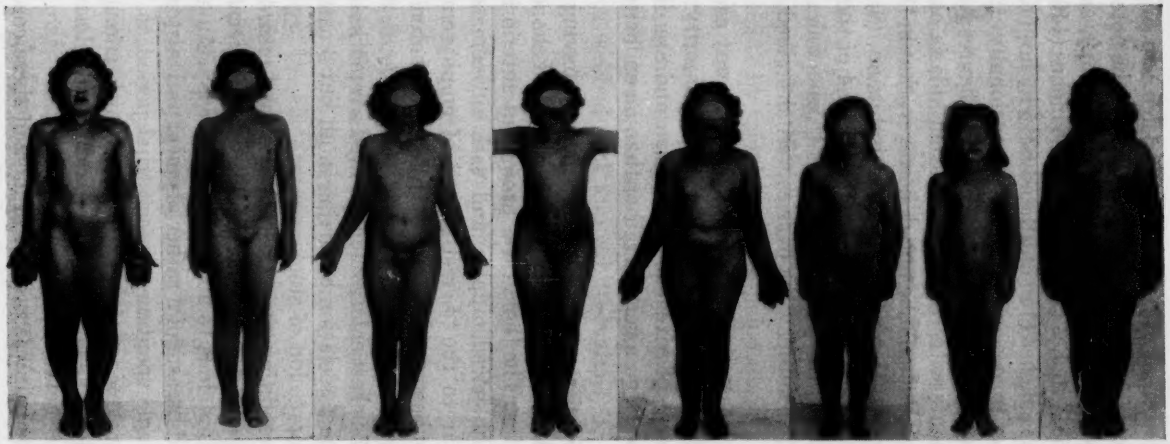
Menstruation	Blood Pressure	Short or Webbed Neck	Cubitus Valgus	Other Congenital Abnormalities	Urinary Gonadotropin Excretion	Bone Age	Osteoporosis	Exploratory Laparotomy or Peritoneoscopy	Additional Data
0	96/40	0	0	0	+192 M.U. −288 M.U.	18	0	0	
0	100/40	+	0	Congenital heart disease.	+288 M.U. −384 M.U.	14	0	0	Looked young
0	132/70	0	+	Rudimentary 1st rib on left. Fusion of 1st & 2nd ribs on rt.	+184 M.U. −308 M.U.	between 21–25	+	0	Has slight tendency to hirsutism, moderate progeria
One scant period act. 17	115/72	+			+384 M.U. −480 M.U.	Adult	0	0	Married "happily" five years
0	140/100	+		Blowing systolic heart murmur.		19–20		0	Looked young. Occasional hot flashes. Mental age 13. BMR +3%
0		+		Excess glial tissue in eyes. Spina bifida occulta		15–16	0	0	
0	110/60	+				At least 22 yrs.	0	0	
0	128/80	0		0		Retarded about 3 yrs.	0	0	Hot flashes from age 15
0	160/100			Coarctation of the aorta. Double left kidney, pelvis, & ureter			+	0	Face looked old, body looked young. Had severe rheumatoid arthritis. Sella "rather small." BMR +9 and +33%. Cholesterol 175
0	158/90	+	+	Madelung's deformity of wrists		21		0	Looked young. Was a teacher. Chest x-ray showed small heart & aorta, but no evidence of coarctation
0	106/50	0	+			14		0	Looked act. 12–13. Bluish sclerae. Deep voice
0	110/75	+	+	Madelung's deformity of wrist joint		Adult		0	Nails fragile, skin and hair dry, slow mental reactions, alopecia areata. BMR −5%
1 scant period 4 mos. before	104/76	+	+			14–15		0	Low pitched voice



	Case 6	Case 13	Case 7	Case 10	Case 11	Case 15
F.S.H.	Age — 27	18	21	39	27	31
Mouse Units	Pos. — 96	192	96	288	165	184
per 24 Hours	Neg.—192	288	192	384	240	308

FIG. 2. Cases with High Urinary Gonadotropins

Patients with abnormally high FSH titers. Of these 5 were verified and are seen also in Figure 1 (*cases 2, 3, 4, 5,* and *6*). The patient on the extreme right differs from the rest in having a history of one scant menstrual period at 17 years of age (see discussion in text under breast development). Note cubitus valgus (exaggerated carrying angle) in *cases 2, 4, 6,* and *15,* and compare with normal appearance of arms in *case 12.* The short webbed neck is clearly noticeable in *cases 4, 9,* and *11.*



		Case 12	Case 3	Case 4	Case 9	Case 2	Case 5	Case 14	Case 16
F.S.H.	Age —	21	15	15	32	29½	13	16	22
Mouse Units	Pos. —	384	80 ±144	197	192	384	27	288	384
per 24 Hours	Neg.—	480	288		288	480	40	384	480
							Normal Less than 5 at this Age		

Another Characteristic Case (8) (No Photos) Positive at 288
Negative at 384

FIG. 2 (*continued*). Cases with high urinary gonadotropins

In this series the height ranged from 47½ inches (120.5 cm.) to 67½ inches (171 cm.) with an average of 55.2 inches (140 cm.). It is to be noted, however, that only 5 of the 25 patients exceeded 57 inches (145 cm.), and their heights were, respectively, 58½ inches (148 cm.), 58–7/8 inches (149.5 cm.), 59½ inches (150.5 cm.), 61¼ inches (156 cm.), and 67½ inches (171 cm.). Interestingly enough, peritoneoscopy in the exceptionally tall patient (*case 6*, Figure 1) revealed an empty pelvis. From these figures it is reasonable to conclude that patients with congenital ovarian aplasia as a rule are under 57 inches (145 cm.). It should be noted, however, that in the literature reviewed and cases reported by Wilkins and Fleischmann, (9) 6 out of 47 cases were found with a height of over 60 inches, and 4 of these were over 62 inches. The tallest was the patient of Pela (3) who was reported to be 70 inches in height; at autopsy the ovaries were described as the "size of orange seeds."

Nevertheless, we do not believe that the ovarian aplasia bears any etiologic relationship to the short stature exhibited by the great majority of these cases. We oppose the concept of sexogenous dwarfism and are disposed to explain the deficient growth on a genetic rather than an endocrine basis.

Sixteen of the 25 patients (64 per cent) were described as having a stocky build. Only 2 were considered thin. A characteristic "shield shaped chest" was another frequent finding. The thorax was prominent anteriorly, broader than normal with widely spaced nipples, and with an increased antero posterior diameter similar to the "barrel chest" of emphysema. This "shield chest", which has not been emphasized heretofore, was noted in 17 of our 25 cases (68 per cent) (especially marked in *cases 1, 2, 4, 11, 18, 22,* and *25*. See Figures 1,2, and 3). The chest enlargement showed a remarkable correlation with stocky build, the association occurring in all but 3 cases. It may emphasize the clinical impression of stockiness.

As would be expected in this condition characterized by sexual retardation, breast development was completely absent in 20 of the 25 cases. Slight development existed in 4 previous to any estrogen therapy. An exception was in the well-developed breasts of a rather atypical patient (*case 16*, Figure 2) who had had a single spontaneous menstrual period at 17 years of age, five years before she came for examination. Curiously enough, her level of gonadotropin excretion was next to the highest of those tested (384 mouse units per twenty-four hours).

A very sparse amount of pubic hair was present in most of these cases. Axillary hair, if present at all, was very sparse indeed. *Case 15* constitutes the single exception. This patient exhibited a constitutional hirsutism, which however, was not heterosexual. It will be noted in her photograph (Figure 2) that her pubic hair was abundant and the axillary hair was also

normal in amount. She had had considerable estrogenic therapy prior to the photograph but claimed that neither the sexual hair nor the slight breast development was influenced thereby. This will be discussed further under therapy.

The external genitalia were invariably infantile, but in 3 instances (*cases 4, 10,* and *16*) the patients were married and claimed satisfactory sexual relations. A third married patient (*case 9*) also reported satisfactory marital relations, but her vagina was found to be so small that normal coitus seemed most improbable. In 1 patient (*case 6*) a plastic operation had been necessary to form even a small vagina. *Case 15* admitted having had intercourse on several occasions, and from the pelvic examination this seemed probable.

As regards the internal genitalia, no adnexa could be felt in any of the patients by rectal or pelvic palpation. In 8 instances the uterus or cervix were described as very tiny.

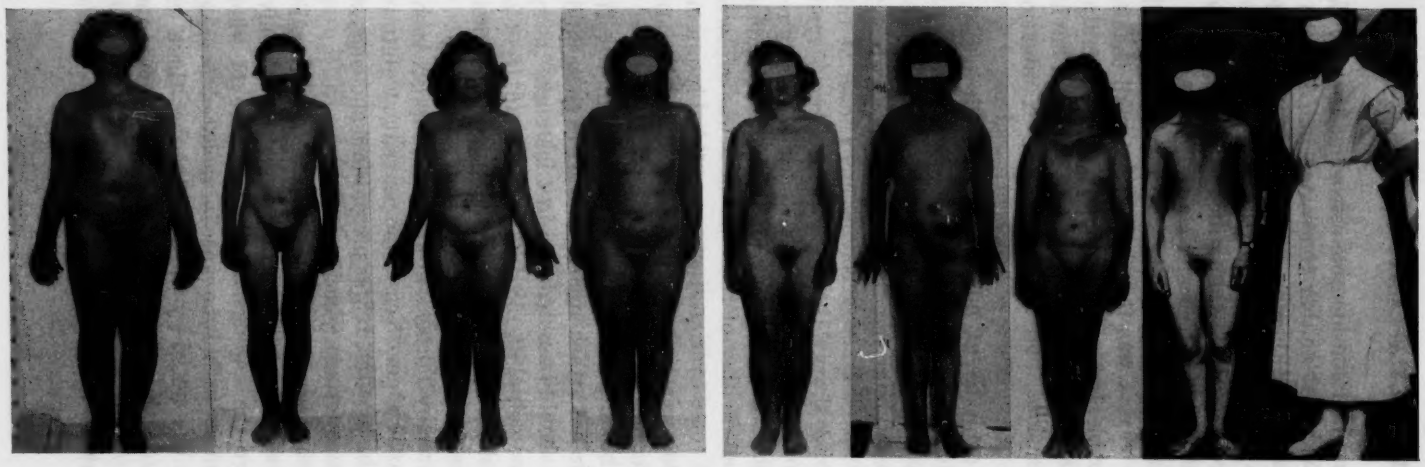
Primary amenorrhea existed in all but 2 of the 25 patients, these 2 each having had a single scant period before receiving any endocrine therapy. One of these (*case 25*, Figure 3) showed the stigmata of Turner's syndrome, with webbed neck and cubitus valgus. The other patient already referred to (*case 16*) had rather well developed breasts and high urinary gonadotropin excretion. Unfortunately neither of these patients was explored, but the evidence suggests that some ovarian tissue was probably present and that a high urinary gonadotropin titer does not necessarily indicate complete absence of gonadal tissue.

In 5 cases the systolic blood pressure was under 100 mm. of Hg, the lowest level being 88. We do not attach any significance to these mildly low levels. On the contrary, it should be noted that in 4 instances the systolic pressure was 140 or over—namely, 140, 158, 160, and 200 mm. Hg. Coarctation of the aorta was proved in only 1 of these. Wilkins and Fleischmann (9) likewise noted hypertension in some of their cases.

Of the stigmata emphasized by Turner, (7) webbing or apparent shortening of the neck was noted in 14 of our 25 cases (56 per cent). Cubitus valgus was specifically looked for in 16 cases. It was present in 9 and suggestive in 1 (total—62 per cent of cases tested) (See Figure 2).

Eleven of the 25 cases showed other congenital abnormalities. Four of these involved the cardiovascular system; coarctation occurred only once. Of particular interest were the findings in *case 1* (Figure 1) where the patient was explored in an effort to find the cause for repeated severe gastrointestinal hemorrhages. It was noted by the surgeon[2] that there were no arterial or venous arcades in the mesentery of the small bowel and the

[2] Dr. Leo Eloesser of San Francisco.



Ages	30	30	16½	21½	18½	16	15½	24
Case	24	19	25	22	20	18	23	17

FIG. 3. Probable ovarian aplasia—no exploratory or FSH. None had menstruated

Eight patients in whom the presumptive diagnosis of congenital ovarian aplasia seemed justified by clinical characteristics. Heights in 7 ranged from 53¾ inches to 58⅞ inches. One had reached 61¼ inches. The majority show a stocky build with the characteristic "shield" chest.

bleeding had presumably occurred from hemangiomata in the intestinal wall. At the time of the exploration he noted also that neither uterus nor ovaries were present in the pelvis. The patient subsequently died, after another severe bowel hemorrhage, the hemoglobin dropping once to as low as 15 per cent. Six of the patients had skeletal abnormalities as follows:—Madelung's deformity of the wrist in 2, osteogenesis imperfecta tarda in 1, anomalous position of a toe in 1, spina bifida occulta in 1, and abnormal development of ribs in 1.

Fifteen of the 25 cases were tested for gonadotropin output (FSH) in the urine.[3] All excreted abnormally high amounts. The method used was a slight modification of that published by Klinefelter, Albright, and Griswold (2). The usual range for adult menstruating females is 10–50 mouse units per day and in children of premenarchial age the range is 0–5 mouse units. Postmenopausal women excrete from 100 to 550 mouse units per day. The lowest titer in our series was 27 mouse units in a child of 13 (*case 5*, Figures 1 and 2) which was well above the normal for that age. Eleven of the remaining 14 were over 150 mouse units with the highest in *cases 2*, *12* and *16*, each of whom excreted over 384 mouse units per twenty-four hours. In the present state of knowledge concerning congenital ovarian aplasia, the finding of a high urinary gonodatropin output would seem to be of paramount importance in establishing the clinical diagnosis, especially in those cases which are somewhat atypical. It should be noted that the gonadotropin excretion value drops to normal levels when the patient is under treatment with estrogens. This is illustrated by our experience in *case 9*. The level on this patient was determined while she was taking stilbestrol 42 mg. monthly and the result was 9.2 mouse units, within normal limits. She was tested again after thirty days without treatment and this time the test was positive for 192 mouse units.

Bone age studies were performed in 24 of the patients and retardation of more than two years was noted in only 4 instances. An additional 7 showed slight retardation, but in the remaining 13, the bone age corresponded to the chronological age. Thus retardation of epiphyseal closure is not the usual finding in this syndrome, which is surprising in view of the striking genital infantilism. In this connection, one should recall that in preadolescent eunuchoidism (in both sexes) some of the epiphyses remain ununited, even after the second decade.

The incidence of osteoporosis in our group was not as high as reported by

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[3] Thanks are due to Dr. Nellie Halliday, director of the Hormone Laboratory, under whose supervision these tests were performed. This laboratory is supported by grants from the Christine Breon Fund and Ciba Pharmaceutical Products, Inc., which are herewith gratefully acknowledged.

other authors. Fleischmann and Wilkins (9) noted its presence in 11 out of 30 cases, whereas our roentgenologists mentioned it in only 5 instances out of 17 with adequate radiologic study. Part of this difference in incidence may be ascribed to varying criteria of what constitutes osteoporosis in x-ray films. The age of the patient does not appear to be a determining factor.

Six of our patients were subjected to peritoneoscopy or exploratory laparotomy (Figure 1). Ovaries were either absent, or represented by vestigial

FIG. 4. Artist's drawing of appearance of pelvis at exploratory laparotomy (performed by Dr. E. W. Overstreet in *case 4*). A. Uterus, infantile in type. B, B. Fallopian tubes, tortuous and rather attenuated. C, C. Rudimentary round ligaments. D, D. "Streak" ovaries. E. Yellowish dots, probably fat in subserosa. November 1942, age 15½.

streaks (Figure 4). The youngest (*case 5*), aged 13, was explored in order to verify the diagnosis so that therapy to promote growth could be instituted well in advance of epiphyseal closure.

A word of caution should be added regarding complete acceptance of peritoneoscopic findings. In *case 2* (Figure 1), where uterus and tubes were stated to be absent, a rudimentary uterus and cervix had been palpated rectally, and the cervix was visualized per vaginum shortly after estrogen therapy was instituted. Another patient (*case 3*, Figure 1) was subjected to peritoneoscopy at 15 and 17 years of age, and no ovaries were seen on

either occasion. However, she menstruated regularly for one year after cessation of estrogen therapy, and subsequently for over three years periods have occurred irregularly at four to nine week intervals without any therapy whatever. Therefore, this patient must have possessed ovarian tissue not recognized by peritoneoscopy.

DIFFERENTIAL DIAGNOSIS

The most important condition to be considered in differential diagnosis is primary pituitary infantilism, which likewise is characterized by shortness of stature and retarded sexual development. The outstanding differential point is the level of urinary excretion of pituitary gonadotropins which is low or absent in pituitary infantilism as contrasted with the decidedly high titer found in ovarian aplasia. There are a number of other distinctions, especially emphasized by Albright, Smith, and Fraser (1), which may be helpful in deciding whether the identical primary amenorrhea, infantile mammae, uteri, and vaginae originate from an anterior pituitary failure or a primary ovarian lack. These differences, however, are more suggestive than convincing; for instance, patients suffering from congenital ovarian aplasia are as a rule short of stature, but not as tiny as pituitary dwarfs. Furthermore, a small amount of pubic and axillary hair is usually present and more can be induced to grow by estrogen therapy, whereas the above authors contend that pituitary dwarfs do not have any sexual hair and none is produced by estrin therapy. One of the characteristic features of the syndrome discussed in this paper is a rather strong well-nourished stocky build with broad deep "shield chest," in contradistinction to the slender gracile Levi-Lorain type of skeleton typical of hypophyseal infantilism. We have previously mentioned the normal or only slightly retarded bone age which differs from the much greater retardation in pituitary dwarfism. Our findings again are in agreement with those of the authors referred to above with respect to the 17-ketosteroid excretion in the urine; this level is apt to be somewhat lower than in a normal female, but not nearly as low as in pituitary dwarfism. Thus, in 7 of our cases the 17-ketosteroid output in milligrams of the ketonic fraction per twenty-four hours was as follows: 1.3 (in a patient aged 13), 4.7, 5.1, 6.1, 6.6, 6.9, and 7.4 mg. (normally menstruating females excrete 6 to 18 mg. per twenty-four hours). Mention should be made of other evidence of congenital confusion such as coarctation of the aorta, webbed neck and cubitus valgus; these have not been reported in association with primary pituitary infantilism. Though none of the above factors can be disregarded, atypical cases occur in both categories, and in such instances especially the most helpful differential "measuring stick" is the urinary gonadotropin level. Of course, peritoneoscopy, or preferably exploratory laparotomy, or finally autopsy,

will confirm the diagnosis, small ovaries being found in pituitary infantilism in contrast to vestigial streaks or complete absence in this syndrome.

Eunuchism or eunuchoidism originating in childhood is more easily differentiated even though the primary amenorrhea and sexual infantilism are closely similar. These girls grow to be fairly tall, in contrast to the short stature characterizing ovarian aplasia. In addition, the typical disproportionately long extremities (eunuchoid proportions) and the prolonged delay in epiphyseal closure help to distinguish the two conditions. This is fortunate since FSH excretion is high in both conditions. For purposes of clinical contrast, a female castrate aged 33 years whose ovaries had to be removed at the age of 5 years is shown in Figure 1.

There should be no difficulty in differentiating the thyroid dwarfism of childhood myxedema; as a rule the shortness is more pronounced and the bone age greatly retarded. The puffiness of the features, obesity, and mental sluggishness are additional distinguishing factors; the sexual status may be retarded but on the other hand may develop precociously—menstruation occurring profusely but irregularly.

The almond-eyed slant and mental retardation of Mongolian idiots should be sufficient to exclude this condition from consideration, even though some of them are short. The typical retroussé nose, long trunk, short extremities, and well developed sexual organs rule out achondroplastic dwarfism from this discussion.

Shortness due to malnutrition, childhood diabetes, renal rickets, or congenital heart disease should not offer any diagnostic difficulties. If necessary, urinary gonadotropin determinations can be resorted to, which of course would be found normal unless ovarian aplasia were associated with one of the above disturbances. It happens that *case 14* in our series had congenital heart disease which may have contributed to her excessively short stature; and *case 10* was complicated by diabetes.

We are of the opinion that a typical case exhibiting primary amenorrhea in association with genital infantilism, short stature, stocky build with shield chest, webbed neck, and cubitus valgus constitutes a syndrome so unique that the assumption of congenital ovarian aplasia seems justified even without corroborative high gonadotropin titers or pathological verification. Unlike some congenital syndromes, notably the Laurence-Moon-Biedl syndrome, the condition is not familial (Figure 5).

It is odd that as yet no comparable condition has been recognized in males.

Finally special allusion must be made to those puzzling atypical instances of verified ovarian agenesis or aplasia where tall rather than short stature is a distracting characteristic. Wilkins and Fleischmann (9) refer to the cases of Meyer, Kuliga, and Pela, the heights being respectively 66.7

inches (169 cm.), 69 inches (175 cm.), and 70 inches (177.5 cm.); the pathological findings concerning the ovaries being "streak," "size of orange seeds," and "none seen during performance of appendectomy." One of our patients (*case* 6, Figure 1) was 67½ inches (171 cm.) tall and as noted peritoneoscopy revealed an empty pelvis. These 4 verified cases of ovarian aplasia, all of them tall, seem to represent a different type and may comprise a separate clinical entity. At the moment their diagnosis, unless

FIG. 5. *Case 4*, aged 15. (Verified) Standing beside normally developed sister age 17, whose height is 65 inches.

substantiated pathologically, may easily be confused with the tall eunuchoids—both types having primary amenorrhea, retarded or absent secondary sex characteristics, and high FSH excretion.

The simultaneous occurrence of congenital anomalies involving, in a definite pattern, such diverse structures as the gonads, skeletal, and cardiovascular systems, is difficult to explain on any but a genetic fault. That the precise location of the defect in a single chromosome (the exact number and relative position of the neighboring genes involved) determines the basic pattern and variations of a given syndrome (4) is an inviting concept.[4] It

[4] This concept was once elaborated by Rados in an attempt to explain the pathogenesis of arachnodactyly, a congenital syndrome involving skeletal, cardiovascular, and ophthalmic systems. It could just as well be extended to apply to other congenital syndromes such as Mongolian idiocy, hypertelorism, and the Laurence-Moon-Biedl picture.

could well explain why, though the majority of patients with ovarian aplasia are short (the basic pattern), some are tall, why some have cardiovascular anomalies and some do not, etc.

THERAPY

The treatment of choice, indeed the only treatment which makes sense and has merit, is the administration of estrogens. The problem which confronts one is the complete or almost complete absence of ovarian tissue. It is clearly indicated therefore that the missing gonadal function must either be replaced or supplemented by substitution therapy.

There can be no point in attempting to stimulate ovarian function by administering anterior pituitary, equine, or chorionic gonadotropins when there is no ovarian tissue capable of responding to such stimulation. Furthermore, the patient's own gonadotropic excretion is far in excess of normal. The ingestion of thyroid substance is equally futile.

In sexually retarded short boys, presumably suffering from pituitary infantilism, treatment with testosterone produces not only sexual advancement, but remarkable stimulation of growth. For this reason, the male hormone has been tried in some cases of ovarian aplasia in the hope of increasing stature. The results have been disappointing, and furthermore, even small doses have produced unpleasant masculinizing effects such as enlargement of the clitoris and huskiness of the voice.

Anterior pituitary pure growth hormone has not as yet been tested on human beings. It will be interesting some day to observe what effect such a preparation would have in adding height to cases of ovarian aplasia.

Having disposed then of all other forms of endocrine therapy, consideration may now be given to the very striking results achieved by administration of estrogens. There are many different preparations available which may be given parenterally or perorally. It is our considered opinion that the latter method possesses the advantages of convenience, economy, and readier regulation. One may choose between the cheaper but also more toxic stilbestrol, and less toxic and yet potent natural estrogens such as ethinyl estradiol (Estinyl, Schering, or Lynoral, Roche-Organon) and conjugated estrogens (equine) (Premarin, Ayerst). The phrase "readier regulation" is used with reference to the "cyclic" method of prescribing oral estrogens, which results in more regular twenty-eight day interval-withdrawal bleedings. The detailed directions in the case of stilbestrol are as follows: 1 milligram each night for one week, 2 milligrams each night for a second week, 3 milligrams each night for the third week, then stop for one week; then start a new cycle as before. If bleeding occurs during administration, stop promptly and resume as above on the third night of flow. Ordinarily this will produce twenty-eight day cycles.

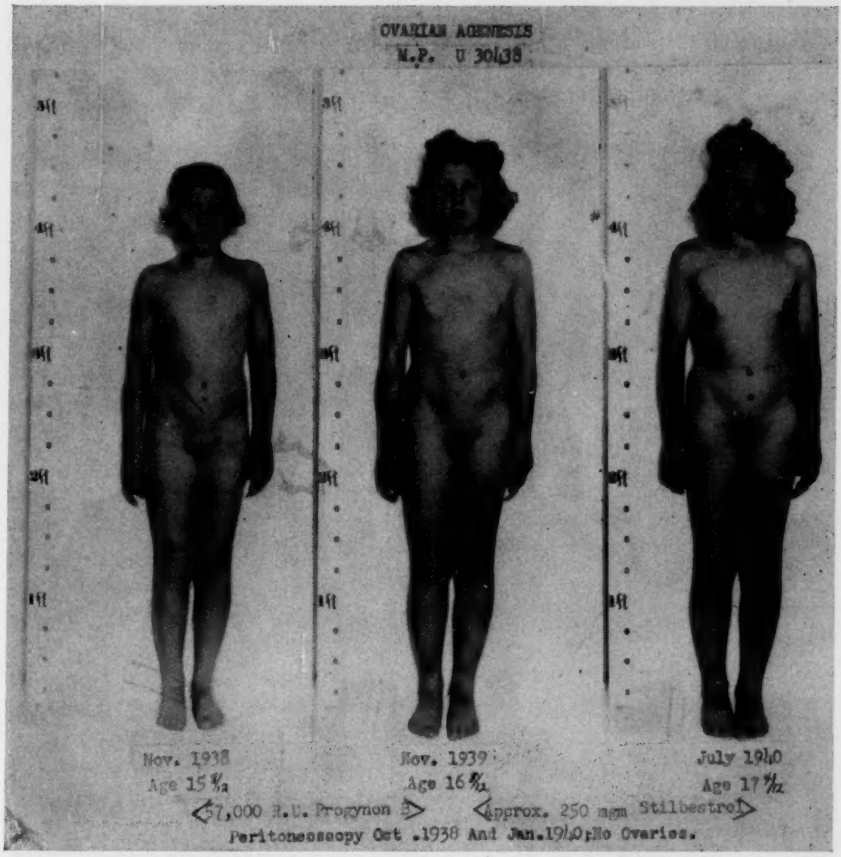

FIG. 6. Results of treatment in *case 3* (verified). Note some breast development, and beginning pubic hair growth under parenteral estrogenic therapy. Note further development with marked nipple areolar pigmentation under stilbestrol given orally. In picture on left, looks fully five years younger than actual age, whereas two years later she looks at least five years older.

Estrin therapy can be counted upon to produce adequate mammary development and growth of sexual hair (Figures 6, 7, 8, and 9). The external and internal genitalia are stimulated to maturity, with the exception, of course, of the absent ovaries. These effects, together with the regular withdrawal bleedings, have a buoyant influence on the morale of the patient, who comes to regard herself as a normally acting and appearing female. This psychic side-effect should not be overlooked or underrated, indeed it is one of the most important and gratifying benefits achieved.

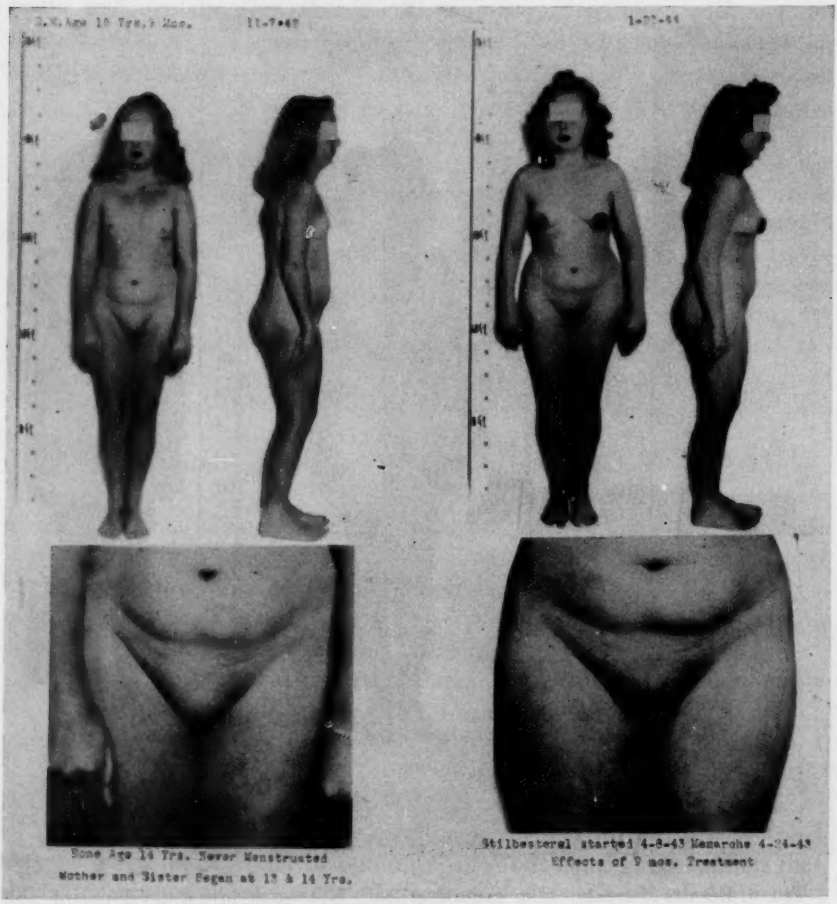
Bone Age 14 Yrs. Never Menstruated
Mother and Sister Began at 13 & 14 Yrs.
Stilbesterol started 4-8-43 Menarche 4-24-43
Effects of 9 mos. Treatment

FIG. 7. Results of treatment in *case 23*, diagnosis of ovarian aplasia based on clinical characteristics. Note growth of pubic hair and striking breast development after nine months of stilbestrol therapy. The enlargement and pigmentation of the nipples were very conspicuous in this patient.

After a year or two of such treatment the patient's sexual status will have been developed to such a degree that marriage is possible and desirable, and normal and satisfying marital relations eventuate. (This happened in *patient 4*, Figure 1.) If marriage is contemplated, the patient of course should be informed that motherhood is not possible.

Though all of the above benefits can, as a rule, be confidently anticipated, very little if any actual stimulation of linear growth is achieved by estrogens. Frankly, nothing noteworthy as regards growth increment was

observed in any of our patients, even though several were under observation for many years. Indeed, unless a powerful growth stimulant is discovered, not much growth can be expected unless the proper diagnosis is made in these patients at an earlier age before the epiphyses are close to

FIG. 8. Breast development in *case 24*, aged 30, resulting from one year of oral stilbestrol therapy.

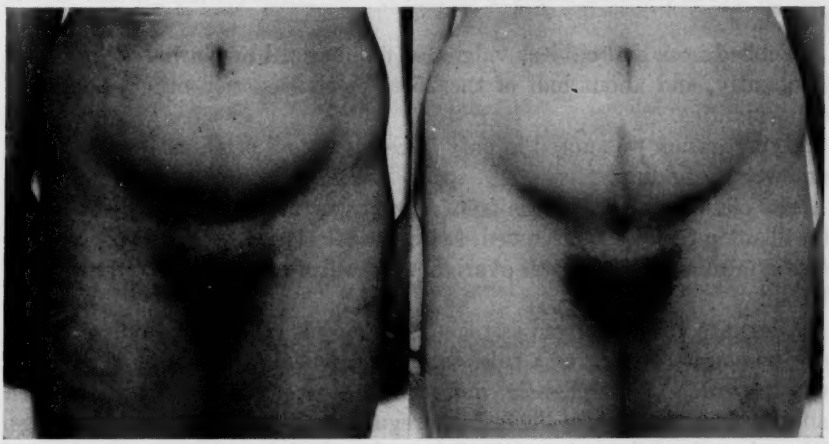

FIG. 9. *Case 4* (verified), aged 15. Note growth of pubic hair from fifteen months' treatment with stilbestrol.

being united. It is to be recalled that the bone age is only slightly less than the chronological age and therefore little opportunity for growth remains if the diagnosis is delayed well into the teen-age.

SUMMARY AND CONCLUSIONS

Twenty-five hitherto unpublished cases of the ovarian aplasia syndrome are presented in tabular form. Six of these have been verified by explora-

tory laparotomy or peritoneoscopy. Fifteen cases were tested for urinary gonadotropin excretion (FSH) and all showed abnormally high titers. Five of these were among the 6 verified pathologically. There remained 9 of the 25 cases in which the diagnosis may be regarded as presumptive, but which we feel justified in including because of the very characteristic clinical picture. This consists of primary amenorrhea in association with genital infantilism, short stature, stocky build with shield chest and sometimes webbed neck, cubitus valgus, and other congenital abnormalities.

Severe sexual infantilism, and of course primary amenorrhea, existed in all but 2 of the 25 cases. One of these exceptions, a girl of 22 years, had well-developed breasts and had had a single spontaneous period five years before.

In only 5 of the 25 patients did the height exceed 57 inches (145 cm.) and only 1 of these was over 61¼ inches (156 cm.) and her height was 67½ inches (171 cm.). As a rule, the stunting of growth was noticed very early in childhood.

Most of the patients displayed a sturdy stocky build with a broad shield-like chest.

Webbed neck and cubitus valgus, as emphasized by Turner, were found frequently, and about half of the 25 cases showed still other congenital abnormalities.

Hypertension was noted in only 5 instances and osteoporosis was not a noteworthy finding.

The clinical differentiation from other types of dwarfism and sexual infantilism in females is discussed. It is particularly important to distinguish this syndrome of congenital ovarian aplasia from primary pituitary infantilism.

Adequate and prolonged estrogenic therapy is specifically indicated and can be counted upon, as a rule, to produce adequate mammary development, growth of sexual hair, more mature external genitalia, and when administered orally in cyclic fashion, regular periodic withdrawal bleedings. These benefits bolster the patient's morale, which is an important aspect of treatment. However, very little growth in height results, partly because the bone age is but slightly retarded.

The recognition of 25 cases from a single clinic suggests that this syndrome is not as rare as suggested by the few cases thus far reported.

SUPPLEMENTARY NOTE

Unfortunately the above article of ours had been set up for printing before the publication of the excellent paper dealing with this same syndrome by E. B. del Castillo, F. A. de la Balze and J. Argonz, entitled "Syndrome of Rudimentary Ovaries with Estrogenic Insufficiency and

Increase in Gonadotropins," which appeared in the June, 1947 number of this Journal. These authors report 8 typical cases of their own, and include a table of 20 similar proven instances published between 1912 and 1941, in other words, prior to the recognition of this new syndrome clinically and hormonically by Varney and collaborators and independently by Albright and co-workers, both publishing their findings in 1942. We regret that this noteworthy contribution from Buenos Aires appeared too late for well deserved comment in our review. Any reader interested in this new syndrome should not fail to consult the above article.

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A CLINICAL EVALUATION OF DIENESTROL, A SYNTHETIC ESTROGEN[1]

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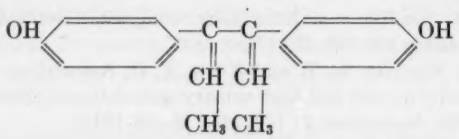
ALTHOUGH the synthetic estrogen, dienestrol, was prepared and described by Dodds and his associates (6) in 1938, it was not until 1942 that Barnes (2) carried out a clinical trial of the estrogen for the inhibition of lactation, reporting in 1944 (3) concerning its effectiveness in the treatment of the menopausal syndrome. The high oral potency of the compound, the absence of untoward or toxic effects, and improved procedures for its preparation have stimulated further clinical investigations of dienestrol.

Dodds, et al. (4, 5), in their description of the preparation of this compound, state that they had occasion to prepare pinacols of p-hydroxyacybenzenes, which on dehydration by means of acetyl chloride and acetic anhydride gave substituted 4:4′-dehydroxydiphenylbutadienes which proved to be powerful estrogenic substances. As in the case of the stilbene derivatives, the maximum activity was found when the central chain comprised six carbon atoms giving a compound 4:4′dihydroxy-γ-δ-diphenyl-β:δ-hexadiene, which was later termed dienestrol, and which can be represented by the following formula:



Dienestrol

In their assay of dienestrol on rats, Dodds et al. (4) found that on subcutaneous injection the compound was slightly less active than diethylstilbestrol which, in turn, they found to be about 2½ times more potent than estrone. They also found the diene to be quite potent on oral administration in oily solution, giving 100 per cent positive results with 3 gamma, while diethylstilbestrol gave 70 per cent positive with 1.0 gamma and 100 per cent with 5.0 gamma. In mice, Emmens (7) found dienestrol a little more potent by mouth in propylene glycol solution than by subcutaneous in-

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jection in oily solution, whereas diethylstilbestrol was only one-fourth as effective by mouth as by injection. He stated that dienestrol had the greatest oral activity, relative to subcutaneous dose, of any estrogen examined up to that time. Noble (10) found that the diene was more potent than diethylstilbestrol in inhibiting the growth of immature rats when implanted under the skin. Andersen (1) tested the potency of dienestrol, diethylstilbestrol, hexestrol and estrone in rats and mice, using single and multiple injections, and vaginal and uterine response as end points, and concluded that the marked differences in relative potency obtained by different methods made it impossible to compare the biologic effectiveness of the different estrogens by such technics.

In a clinical study of the inhibition of lactation by synthetic estrogens, Barnes (2) treated 57 patients with dienestrol, 81 with stilbestrol and 16 with hexestrol. She concluded that dienestrol gave successful results with as little as one-tenth of the dose required for stilbestrol or hexestrol. The most satisfactory results were obtained with an initial dose of 0.5 mg. twice daily, decreasing the dose by 0.1 mg. each day down to 0.1 mg. twice daily. All three synthetic estrogens were well tolerated by the puerperal women.

Barnes (3) also found dienestrol given by mouth to be highly effective in the treatment of menopausal symptoms in a group of 11 patients. With a dosage of 0.1 mg. twice daily for a period of four weeks or more, hot flashes were relieved or cured in 7 of 9 cases. Higher dosages were not tried in the two cases which failed to respond. Senile vaginitis, which was a symptom in four cases, was relieved or cured in all. None of these patients experienced nausea, vomiting or other symptoms of intolerance, and only one patient had vaginal bleeding. Vaginal smears were made once every four weeks; in 3 instances a definite estrogenic response was obtained; in 3 instances the atrophic changes in the smear were not sufficiently marked to determine a change, and in 5 instances the changes were too indefinite to warrant a conclusion.

Finkler and Becker (8) have recently reported a group of 62 menopausal patients treated with dienestrol for a period of 6 weeks to 6 months with daily doses of 0.2 to 1.5 mg. They, too, noted prompt relief of menopausal symptoms, and that untoward effects were minimal and withdrawal bleeding infrequent. No analysis was made of the minimal dosage necessary to produce a clinical response. Improvement in vaginal smears usually paralleled clinical improvement, although in a few cases the latter was achieved without any marked changes in the vaginal smear. Slight nausea was noted in only 3 cases, and in 2 of these there was some question as to whether it was due to the medication. Mild withdrawal bleeding occurred in only two instances. In a further report (9), bringing their menopausal cases to a

total of 70, no additional cases of bleeding or intolerance were noted. Sikkema and Sevringhaus (11) employed dienestrol in 21 patients and reported no nausea, emesis, nor other unpleasant side reactions with dosages up to 0.5 mg. twice daily. In 13 climacteric women who were adequately followed, satisfactory relief of symptoms was obtained with doses of 0.2 to 0.5 mg. On the basis of this series they stated that dienestrol was the most satisfactory synthetic estrogen with which they had had experience. In contrast to Finkler and Becker (8), they did not note the sense of "well-being" with dienestrol which followed some of the natural estrogens.

PURPOSES AND METHODS OF PRESENT STUDY

Our clinical investigations with dienestrol were undertaken with the following objectives: 1) to determine the relative usefulness of dienestrol

TABLE I. CONDITIONS TREATED—82 PATIENTS

Menopausal Syndrome	40
Postpartum Lactation	26
Secondary Amenorrhea	5
Cystic Mastitis	4
Atrophic Vaginitis	3
Ovarian Agenesis	2
Adrenogenital Syndrome	2
Premenstrual Migraine	2
Pseudocyesis	1
Hypomenorrhea	1
Cystic Disease of Ovaries	1
Premenstrual Epistaxis	1
Essential Dysmenorrhea	1

for the various conditions in which estrogenic therapy is indicated; 2) to evaluate the estrogenic response by objective findings such as on vaginal smear response, occurrence of withdrawal bleeding and, in some instances, hormone assays; 3) to determine any possible toxic effects by repeated clinical and laboratory studies in patients taking the hormone over prolonged periods of time.

Dienestrol was administered routinely to all patients requiring estrogenic therapy as they appeared in the clinic. In each instance before treatment was undertaken the patient was thoroughly studied by physical examination, including a pelvic examination, urinalysis, blood count, vaginal smears, and whatever other laboratory procedures were indicated to establish a definite diagnosis, including in many instances assays of urinary gonadotropins, 17-ketosteroids and estrogens. In a group of twenty-five unselected patients in whom prolonged therapy was planned or anticipated, the following studies were made and repeated at intervals of one to

three months in order to determine any possible toxic effects on the blood, kidneys or liver: complete blood count, urinalysis, bromsulfalein liver function test, serum bilirubin, urine urobilinogen, cephalin flocculation and thymol turbidity test. Vaginal smears for evaluation of estrogenic effect were made at each visit. Treatment was started with a minimal dosage of hormone, such as 0.1 mg. daily by mouth, which was increased at weekly intervals until the effective dosage was reached; they were re-examined at intervals of 2 to 4 weeks. Postpartum patients who received dienestrol for inhibition of lactation were treated in the obstetric wards and, when necessary, were followed in the postnatal clinic. Ninety-six patients were started on treatment with dienestrol, 82 of whom were adequately followed; the conditions for which they were treated are indicated in Table I.

RESULTS

Menopausal Syndrome: In patients with a typical menopausal syndrome dienestrol was given in slowly increasing dosage in order to determine 1) the minimal dosage necessary to obtain complete or nearly complete relief from the hot flashes and related vasomotor symptoms, 2) the minimal dosage required to change the vaginal smear from an estrogen deficiency type to one showing a slight estrogen effect, in which practically no basal layer cells are seen but the squamous epithelial cells are of the precornified type, 3) the dosage necessary to produce a good or marked estrogen effect in the smear, in which the squamous epithelial cells are chiefly of the cornified type. The results, related to the status of the patient at the onset of therapy, are indicated in Tables II, III, and IV.

TABLE II. MENOPAUSAL SYNDROME—CLINICAL RESPONSE

	Dosage Necessary for Symptomatic Relief				
	0.1 mg.	0.2 mg.	0.3 mg.	0.5 mg.	1.0 mg.
Severe Cases (13 Patients)	0	2	0	8	3
Moderate Cases (20 Patients)	4	3	0	12	1
Mild Cases (7 Patients)	3	1	1	2	0
Entire Group) (40 Patients)	7	6	1	22	4

TABLE III. OVARIAN DEFICIENCIES—SMEAR RESPONSE

	Dosage Necessary to Correct Vaginal Smear to Slight Estrogen Effect						
	0.1 mg	0.2 mg.	0.5 mg.	1.0 mg	1.5 mg.	2.0 mg.	3.0 mg.
Marked Deficiency (11 Patients)			2	7	2		
Moderate Deficiency (13 Patients)			5	4		3	1
Slight Deficiency (5 Patients)	1	1	3				
Minimal Estrogen Effect (11 Patients)	3	6	2				
Entire Group (40 Patients)	4	7	12	11	2	3	1

TABLE IV. SMEAR RESPONSE ON HIGH DOSAGE

	Dosage Necessary to Produce Good Vaginal Cornification			
	0.5 mg.	1.0 mg.	2.0 mg.	3.0–6.0 mg.
Marked Estrogen Deficiency			1	1
Moderate Estrogen Deficiency	1	1	2	1
Slight Estrogen Deficiency			1	2
Minimal Estrogen Effect	6		2	
Entire Group Tested (18 Patients)	7	1	6	4

It will be noted (Table II) that slightly more than half of the patients required 0.5 mg. of dienestrol to bring their symptoms under control but that the majority of the patients with mild symptoms and about a third of those with moderately severe symptoms could be controlled with 0.1 to 0.3 mg. daily. Only 4 patients (10 per cent) required a dosage of 1.0 mg. to control their symptoms. Improvement was generally noted within five days

but it is to be emphasized that many patients, as is the case with other orally effective estrogens, did not receive maximum benefit for 2 to 3 weeks, so that it is neither wise nor necessary to increase the dosage too rapidly.

The dosage necessary to obtain a vaginal smear response was in most instances considerably higher (Table III) than the dosage necessary to obtain a good clinical response. Seventeen (43 per cent) of the patients required 1.0 mg. or more to obtain a minimal estrogen effect, one patient requiring as much as 3 mg. The average dosage required to obtain a vaginal smear response in the entire series was 0.77 mg., while a good clinical effect was obtained with an average dosage of 0.43 mg. daily.

In 18 patients (Table IV) the dosage was gradually increased further to determine the minimum amount necessary to obtain a marked estrogenic effect on vaginal smear, as indicated by the presence of large numbers of cornified cells. In patients who started with a minimal estrogen effect, i.e., chiefly precornified cells, it usually required 0.5 mg. daily to obtain a full effect; in patients with initial estrogen deficiency smears, considerably higher dosages were required, up to 6.0 mg. daily in one case. The average dosage for this group was 1.8 mg. daily, or about four times the average amount necessary to obtain a clinical response. In individual cases this ratio was as high as 12:1.

The improvement in other symptoms associated with the menopausal syndrome, which are more difficult to evaluate, such as nervousness, irritability, headache, depression, arthralgia, etc., paralleled our experience with other estrogens given in doses which afforded relief from the vasomotor symptoms. There were 3 patients in this group with symptoms attributable to a marked atrophic vaginitis. These patients required daily 1.0, 2.0 and 3.0 mg., respectively, to obtain relief and satisfactory vaginal cornification. The dosage was considerably higher than that which they required for improvement in their systemic manifestations. Six other women with atrophic vaginitis not associated with other symptoms were treated with dienestrol locally, using a vaginal cream containing 0.1 mg. dienestrol per cc. With the daily intravaginal administration of 5 cc. (0.5 mg.) excellent therapeutic and vaginal smear response was obtained within a week.

Dienestrol was very well tolerated by all menopausal patients. None complained of nausea, vomiting or other gastro-intestinal symptoms which could be attributed to the drug, nor were there any other evidences of intolerance sometimes noted with other synthetic estrogens, such as increased headache, vertigo, etc. One patient developed a skin rash soon after starting dienestrol, which persisted, however, after withdrawal of the drug and was thought to be a neurodermatitis.

Despite the prolonged period of treatment of most of these patients,

uterine bleeding during therapy or on withdrawal was uncommon (Table V). It occurred in only 2 patients (5 per cent) who were receiving therapeutic dosages of dienestrol, and in 2 patients who were receiving increased dosages of dienestrol for the purpose of testing the vaginal smear response. It will be noted (Table V) that in three of these patients irregular bleeding had occurred prior to dienestrol therapy, either of functional origin or following administration of other estrogens. Patients of this type are not good candidates for estrogen therapy. Further bleeding always arouses the suspicion of malignancy and often requires diagnostic curettage to rule out this possibility with certainty. This procedure became necessary in two of

TABLE V. BLEEDING IN MENOPAUSAL WOMEN WITH DIENESTROL

Patient	Clinical Status	Dienestrol Therapy Preceding Bleeding	Remarks
R.G. Age 46	Mild menopausal syndrome	0.1 mg. daily for 6 months	Previous irregular bleeding on occasion. D & C negative
M.M. Age 32	Surgical castrate, supravaginal hysterectomy, severe menopausal syndrome	0.5 mg. daily for 6 weeks	Previous bleeding with premarin 1.25 mg. and stilbestrol 0.5 mg.
M.F. Age 48	Severe menopausal syndrome following radium for functional bleeding	0.1 to 0.5 mg. for 6 months. Gradually increased to 2.0 mg. over additional 3 months	Subsequent D & C showed scant curettings
A.T. Age 60	Severe menopausal syndrome for 6 years	0.1 to 0.2 mg. for 5 months, 0.5 to 1 mg. for 2 months	Slight spotting after withdrawing dienestrol

these patients (*R.G.* & *M.F.*); following further spotting three months later the latter patient had a second curettage which showed an atrophic endometrium.

Inhibition of Lactation: Dienstrol was administered to 26 postpartum women in whom it was desired to inhibit lactation. These women were 1 to 5 days postpartum when treatment was started. They received dienestrol in a dosage of 0.5 mg. twice daily for 3 days and then 0.5 mg. daily for approximately 1 week. The results were uniformly good. In patients treated immediately postpartum, the breasts did not fill and lactation was inhibited, while in those started later engorgement subsided promptly and the breasts dried up during the course of treatment. None of the pa-

tients exhibited any evidences of intolerance to dienestrol, nor was there a single instance of postnatal hemorrhage. When seen in the postpartum clinic subsequently, resumption of menstrual function had occurred within the usual time.

Amenorrhea: Dienestrol was used in the treatment of amenorrhea in nine young women, the amenorrhea being associated with a syndrome suggesting ovarian agenesis in two cases, adrenogenital syndrome in two cases, pseudocyesis in one case, and secondary amenorrhea due to psychogenic or other factors in four cases. The pertinent data in these cases are as follows:

1. *J.K.* Age 22. Syndrome suggestive of ovarian agenesis. Studies showed an estrogen deficiency smear and high urinary gonadotropins. She received dienestrol 1 mg. daily for 6 months. The breasts enlarged and there was moderate development of secondary sex characters. The vaginal smear improved from an estrogen deficiency to a good estrogen effect. A blood tinged discharge was noted at times, but no frank bleeding occurred even on withdrawal of dienestrol.

2. *H.B.* Age 25. This was a typical case of ovarian dwarfism, previously treated with other estrogens. Cyclic administration of dienestrol 1 mg. daily plus progesterone, 20 mg. by injection twice weekly at the end of the cycle, resulted in cyclic bleeding, but dienestrol alone did not produce this result. The results were comparable to those previously obtained in this patient with stilbestrol or estradiol plus progesterone, except that it was possible to obtain withdrawal bleeding at times with stilbestrol alone in a dosage of 0.25 mg. daily by mouth for a month.

3. *F.P.* Age 21. This patient had an adrenogenital syndrome characterized by hirsutism, impaired glucose tolerance, secondary amenorrhea for 3 years, sterility, and moderately increased urinary 17-ketosteroids. Cyclic bleeding was induced with 0.5 mg. dienestrol plus 20 to 60 mg. progesterone, by injection, at the end of the cycle. This was continued for 6 months. Soon after treatment was discontinued this patient became pregnant and carried successfully to term.

4. *E.A.* Age 35. An adrenogenital syndrome was present associated with secondary amenorrhea for 10 years, hirsutism, impaired glucose tolerance, and increased urinary 17-ketosteroids. Bleeding was induced with dienestrol 1.0 mg. daily plus progesterone given at the end of cycle in a dosage of 20 to 60 mg. Subsequently, dienestrol was effective alone, in a dosage of 0.5 mg. daily.

5. *C.R.* Age 32. Amenorrhea in this patient was associated with pseudocyesis and was followed by symptoms of a menopausal syndrome. Vaginal smear showed an estrogen deficiency and the urinary gonadotropin value was high. One mg. of dienestrol daily was necessary to give relief from hot flashes, but bleeding could not be induced even with a dosage of 2 mg. daily for 3 months and the patient still had an estrogen deficiency smear.

6. *M.M.* Age 35. Secondary amenorrhea was present for 7 years, with no associated symptoms. Vaginal smear showed an estrogen deficiency and the urinary gonadotropin value was high. Estradiol benzoate, 10,000 rat units, plus progesterone, 20 mg. daily, each given for 3 days by injection, produced bleeding. Dienestrol, 1 mg. daily by mouth, followed by progesterone in the above dosage for one month produced no bleeding. Stilbestrol, 1 mg. daily by mouth, followed by progesterone, resulted in vaginal bleeding. Dienestrol and progesterone were again tried with no results.

7. *S.S.* Age 34. Premature menopausal syndrome with amenorrhea and hot flashes

of 5 years' duration. Stilbestrol 0.5 mg. daily afforded good relief from symptoms; bleeding could be brought on cyclically by adding progesterone late in the cycle in a dosage of 20 to 40 mg. Equal relief from symptoms was obtained with dienestrol, 0.3 mg. daily, but it was not possible to induce bleeding by adding progesterone as above.

8. *J.C.* Age 33. Secondary amenorrhea and mild hot flashes for 2 years following resection of an ovarian cyst. Vaginal smear showed an estrogen deficiency and the urinary gonadotropin value was high. Symptoms were well controlled with dienestrol 0.1 mg. daily. No bleeding occurred when the dosage was increased to 0.5 mg. and given cyclically, but was readily induced by adding progesterone, 20 mg. at the end of cycle.

9. *Y.E.* Age 28. Secondary amenorrhea of 6 years' duration, associated with hot flashes, an estrogen deficiency smear and high urinary gonadotropin. Dienestrol, 0.5 mg. daily, given in cyclic fashion produced symptomatic relief but no withdrawal bleeding. Stilbestrol, 0.5 mg. daily, produced symptomatic relief and resulted in cyclic spotting or bleeding.

Other Conditions: One patient with essential dysmenorrhea received dienestrol for inhibition of ovulation. A successful result, as judged by absence of pain, was obtained the first month with a dosage of 1 mg. daily during the first 21 days of the cycle; the second month, moderate dysmenorrhea occurred with this same dosage, but successful results were obtained the third month with a dosage of 2 mg. daily. It has been our experience with other estrogens that patients with essential dysmenorrhea frequently require increasing doses of estrogen to maintain inhibition of ovulation.

Four patients with cystic mastitis had previously shown improvement with cyclic administration of other orally effective estrogens. Comparable improvement occurred with dienestrol in dosages ranging from 0.2 to 1 mg. daily.

A patient with premenstrual epistaxis was a young woman, age 25, with a normal 28 day menstrual cycle, who was treated with dienestrol in an attempt to inhibit menstruation. One mg. daily for two months failed to delay the period, but inhibition was obtained, with improvement in the epistaxis, with 2 and 3 mg. of dienestrol daily. Normal cycling followed withdrawal of the hormone and the patient became pregnant the following month. This woman kept basal temperature charts during the course of dienestrol therapy. These showed absence of the usual sharp ovulatory rise during the period of dienestrol therapy, with a sharp ovulatory rise in the month when therapy was withdrawn and pregnancy occurred.

Only moderate increase in menstrual flow was noted in a patient with hypomenorrhea treated with dienestrol, 1 mg. daily in cyclic fashion. In two patients with premenstrual migraine, the results of dienestrol therapy were inconclusive, as was the response to other estrogens. Improvement in pain and menstrual irregularity occurred in the one patient with cystic disease of the ovaries (associated with high gonadotropin excretion), treated with dienestrol, 0.5 mg. daily, in cyclic fashion.

Toxicity Studies: None of the patients receiving dienestrol exhibited any clinical evidence of toxic effects attributable to the drug, nor were any patients intolerant to the medication. Nausea, vomiting or headache, which are common symptoms of intolerance to some estrogens, were not encountered. As previously mentioned, there was only the one instance of skin rash and this was probably not caused by the drug.

The laboratory studies in the twenty-five patients receiving protracted dienestrol therapy also proved negative for any toxic effects. These patients received dienestrol continuously in doses of 0.1 to 3 mg. daily (average 0.82 mg.) for periods ranging from 4 to 16 months (average 7.3 months). The patients ranged in age from 18 to 65 years and included various syndromes of ovarian deficiency. The following complications were also present: thyrotoxicosis in 3 cases, essential hypertension in 4 cases, arthritis in 2 cases, functional hepatic impairment in 1 case, diabetes in 1 case, pyelitis in 1 case, duodenal ulcer in 1 case, hayfever in 1 case. No significant alterations were noted in the hemoglobin, erythrocytes, or leucocytes during the course of treatment. Urinalysis also failed to show any significant changes. In 24 patients beginning treatment with normal liver function no significant alterations were noted in bromsulfalein excretion, serum bilirubin, or urine urobilinogen. One menopausal patient with a history of previous hepatitis started treatment with 5 per cent retention of bromsulfalein at 30 minutes, a serum bilirubin of 0.4 mg. per cent and slight increase in urine urobilinogen. During the course of treatment with dienestrol (0.2 to 0.5 mg. per day) these values remained essentially the same; at the last examination after 10 months of treatment the bromsulfalein test showed dye retention of 5 per cent at 30 minutes, the serum bilirubin was 0.7 mg. per cent and the urine urobilinogen was unchanged. Cephalin-cholesterol flocculation decreased in one patient from +4 to +2, and in 3 others from +2 to 0. It increased in one case from +1 to +3 with no other abnormality, and remained at +3 in one other case. The thymol turbidity test in the latter case increased from 5.2 to 8.2 while on treatment with dienestrol, 1.0 mg. for 7 months. The bromsulfalein test in this patient remained normal, urine urobilinogen fell to normal from slightly increased values, and serum bilirubin remained at 0.2 mg. per cent.

DISCUSSION

Although many estrogenic substances are available for clinical use, none is ideal for all purposes. The choice of an estrogen depends upon many factors, including the physiologic effects desired, the route of administration, tolerance, toxicity, untoward effects, and cost. In an evaluation of any new estrogenic substance all of these factors deserve consideration.

Physiologic Effects: From the studies thus far reported dienestrol ap-

pears to be of particular value for the control of the vasomotor and related symptoms of the menopausal syndrome and for inhibition of lactation, whereas it is less effective than diethylstilbestrol if a proliferative effect on the endometrium or vaginal mucosa is desired. These observations suggest that dienestrol has a relatively marked inhibitory effect upon the pituitary as compared to its influence upon the end organ. This is also suggested by the findings of Noble (10), who noted that dienestrol had a stronger inhibiting effect than diethylstilbestrol upon the growth of immature rats. In a number of preliminary experiments in castrates and menopausal women we have been able to inhibit excessive gonadotropic excretion in some but not in all instances. This point is being studied further.

Route of Administration: Dienestrol appears to be unique in that its oral potency is as great as, if not greater than, its potency by injection when tested in the mouse and the rat, as reported by the English workers. We have been able to confirm this observation in the mouse in our laboratory. In some preliminary observations in castrates and lactating women we have found dienestrol to be effective when given parenterally in oil or in aqueous suspension but not as effective as similar doses given by mouth. When given by injection or by mouth, dienestrol does not appear to have a prolonged action, such as has been noted with monomestrol when given by injection. In mice and rats we have found that estrus lasts for an average of 3 days following a parenteral or oral dose of ten times the minimal estrogenic dose. Preliminary studies of the metabolism of dienestrol have shown that when injected into bile-fistula dogs a considerable proportion of the estrogenic activity (more than 20 per cent) can be recovered in the bile. No observations are as yet available on the blood concentration or urinary excretion following its administration.

Dienestrol is apparently active when applied locally to the vaginal mucosa of humans, as indicated by the excellent cornification obtained in patients with atrophic vaginitis. Its relative efficacy in this respect as compared to other estrogens remains to be determined.

Tolerance: It has been the consensus of several clinical observers that dienestrol is one of the best tolerated of all estrogens. It may be given in full dosage with but little fear of nausea, vomiting, headache, skin rash or other symptoms. Our studies indicate that even when given over a long period of time there are no significant effects on the blood, kidneys or liver or any other evidence of toxicity.

Although bleeding on withdrawal or during the course of dienestrol therapy has been significantly less in menopausal women treated with dienestrol than with other estrogens, it has occurred in a sufficient number of instances in our own experience and in the reports of others to indicate

that this is the chief untoward effect to be guarded against. Because dienestrol is so well tolerated and because withdrawal bleeding is reported as uncommon there is a real danger that dienestrol will be administered too freely and in too high dosage to menopausal women, with the result that withdrawal bleeding will be induced. In this study we showed that although bleeding occurred only twice in 40 patients with minimal effective therapeutic dosages it was induced in 2 more of 18 patients by further increasing the dosage. Once such bleeding begins the fear of malignancy is raised and frequently diagnostic curettage is necessary to rule it out. It is still essential to follow the rule of keeping patients on the minimal effective dosage and to withdraw therapy as soon as feasible.

Cost: Improved methods in the synthesis of dienestrol have made its commercial production less expensive than by the original process described by Dodds and his associates (4, 6). Dienestrol has therefore become available as a relatively inexpensive estrogen.

CONCLUSIONS

Dienestrol is a potent estrogen which is highly effective when given orally. It is more potent than diethylstilbestrol for inhibiting lactation. It is of approximately the same range of potency as diethylstilbestrol for the control of the vasomotor symptoms of the menopause. It is less effective then diethylstilbestrol in inducing marked estrogenic response of the endometrium or vaginal epithelium. Withdrawal bleeding does not often occur with commonly used doses of dienestrol. Even in high dosage, dienestrol is well tolerated when given for prolonged periods of time and does not give rise to toxic effects. Dienestrol is considered to be a particularly useful estrogen for the treatment of the menopausal syndrome or for the inhibition of lactation.

SUMMARY

The clinical response to dienestrol (4:4′ dihydroxy-γ-δ-diphenyl-β:δ-hexadiene) was evaluated with reference to symptomatic improvement, objective response, and evidences of toxicity in 82 patients with various conditions in which estrogen therapy was indicated.

In 40 menopausal women, dienestrol afforded excellent relief in every instance, the minimal required dosage ranging from 0.1 to 1.0 mg., with an average of 0.43 mg. daily. However, the average dosage required to transform the deficiency smear to one showing slight estrogen effect was 0.77 mg., while the production of a marked estrogen effect required daily doses up to 6.0 mg. (average 1.8 mg.). Bleeding during the course of treatment or on withdrawal occurred in only two patients on ordinary therapeutic dosages and in two other patients with higher dosages.

On local application to the vagina in 6 cases of atrophic vaginitis good

results were obtained with a vaginal cream containing 0.1 mg. per cc. (0.5 mg. per dose) while in 3 similar cases doses of 1 to 3 mg. were required by mouth.

In 9 younger women with primary or secondary amenorrhea associated with primary ovarian deficiency it was difficult to induce withdrawal bleeding even with a relatively high dosage of dienestrol, which is considerably less effective than diethylstilbestrol in this respect.

In 26 postpartum patients, inhibition of lactation and breast engorgement was readily obtained with a dosage of 1.0 mg. daily for 3 days and 0.5 mg. thereafter for one week.

These observations suggest that dienestrol, given orally, has a relatively marked inhibitory effect on the pituitary and a relatively weaker estrogenic effect on the endometrium and vaginal mucosa.

No symptoms of intolerance to dienestrol were noted in any patient even in high dosage. In 25 patients treated for 4 to 16 months (average 7.3 months) no evidence of toxicity was noted nor any significant changes in blood count, urinalysis, or liver function tests.

It is concluded that dienestrol is a particularly useful estrogen for the treatment of the menopausal syndrome and for the inhibition or suppression of lactation.

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A SIMPLE QUANTITATIVE COLORIMETRIC METHOD FOR ESTROGENIC STEROIDS

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MANY colorimetric methods for determining natural estrogens have been described, most widely used of which is the reaction described by Kober (1931) (4). Although supposedly specific for estrogenic steroids, this reaction has been shown by Mather (1940) (6) to give similar positive tests with five times as much androsterone.

Kober (1931) (4) noted that a red color was obtained upon the addition of concentrated sulfuric acid to estrone, heating, diluting with water and reheating. However, an intense greenish fluorescence was also present which interfered with the quantitative colorimetric determination by visual methods. He found that addition of phenol quenched the fluorescence and permitted a more accurate determination of the red color.

The Kober test is a two phase reaction. The first is heating with phenolsulfonic acid, which produces a yellow colored solution, and the second phase is dilution with water or dilute sulfuric acid and reheating, which produces a red colored solution. Various modifications have been described by Cohen and Marrian (1934) (3), Cartland et al. (1935) (2), Pincus et al. (1936) (7), Venning et al. (1937) (9), Kober (1938) (5), Bachmann (1939) (1) and Szego and Samuels (1943) (8).

In the present work a re-evaluation of the reaction with concentrated sulfuric acid has been carried out and conditions set forth in which an optimum red color is obtained using only sulfuric acid. The greenish fluorescence, although present, has been found to offer no interference when photoelectric colorimeters are utilized.

MATERIALS AND METHODS

Crystalline estrone (m.p. 254–256°C.), estriol, and α-estradiol were used. All colorimetric values were obtained with a Beckman spectrophotometer. The reagents used were sulfuric acid, c.p., and absolute ethanol. All heatings were performed in a boiling water bath. No particular precautions were taken to maintain anhydrous conditions during the heating periods.

All dilutions of sulfuric acid are expressed on a volume to volume basis with the volume of sulfuric acid indicated first, e.g., (90+10) means 90 ml. of concentrated sulfuric acid was mixed with 10 ml. of distilled water.

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EXPERIMENTAL RESULTS

1. Effect of Sulfuric Acid Concentration at Time of Second Heating:

A solution of 800 mg. of crystalline estrone dissolved in 2 cc. of absolute ethanol was heated with 20 cc. of sulfuric acid, c.p., for 2 minutes at 100°C. Two cc. aliquots of this solution were diluted with 10 cc. of the indicated dilutions of sulfuric acid, reheated for 3 minutes at 100°C., cooled and absorption curves obtained (Fig. 1). These curves demonstrate the diphasic

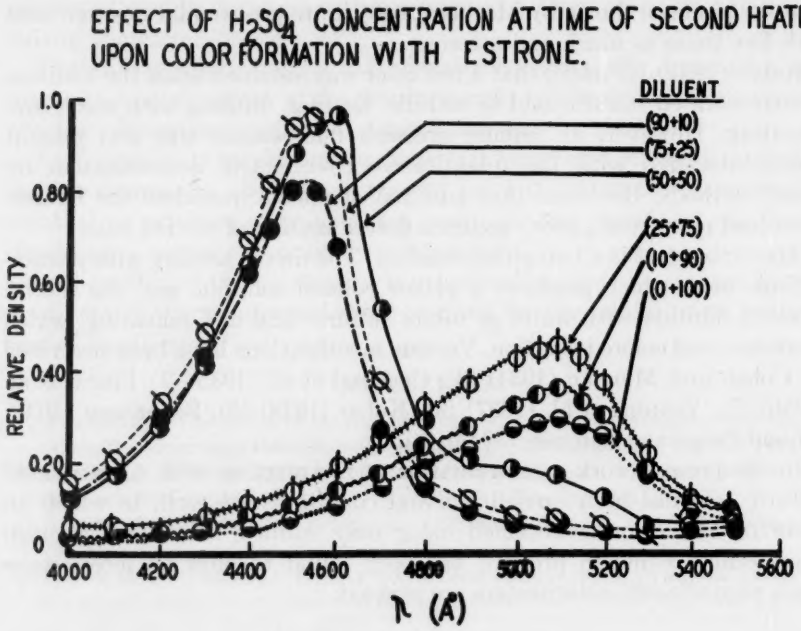

FIG. 1

nature of the reaction. With sulfuric acid (90+10) as the diluent for the second heating a maximum is obtained at 4520 Å with little or no absorption in the green region. As progressively more dilute sulfuric acid solutions are used, the absorption in the blue region decreases, and absorption in the green increases. With sulfuric acid (25+75) the maximum absorption occurs at 5100 Å while the absorption at 4520 Å has dropped to a minimum. More dilute sulfuric acid (10+90) and distilled water when used as diluent for the second heating, yield curves similar to those obtained with sulfuric acid (25+75) but the intensity of the red color is reduced. Thus maximum red color formation is obtained by using sulfuric acid (25+75) for dilution before the second heating.

2. Effect of Duration of First and Second Heating Upon Color Density:

Since the reaction is essentially a two phase phenomenon the conditions necessary for obtaining maximum color development were studied by varying the times of first and second heating. Samples (0.4 cc. each)of alcoholic solutions of estrone and estriol containing 40 μg. were treated with 2 cc. of

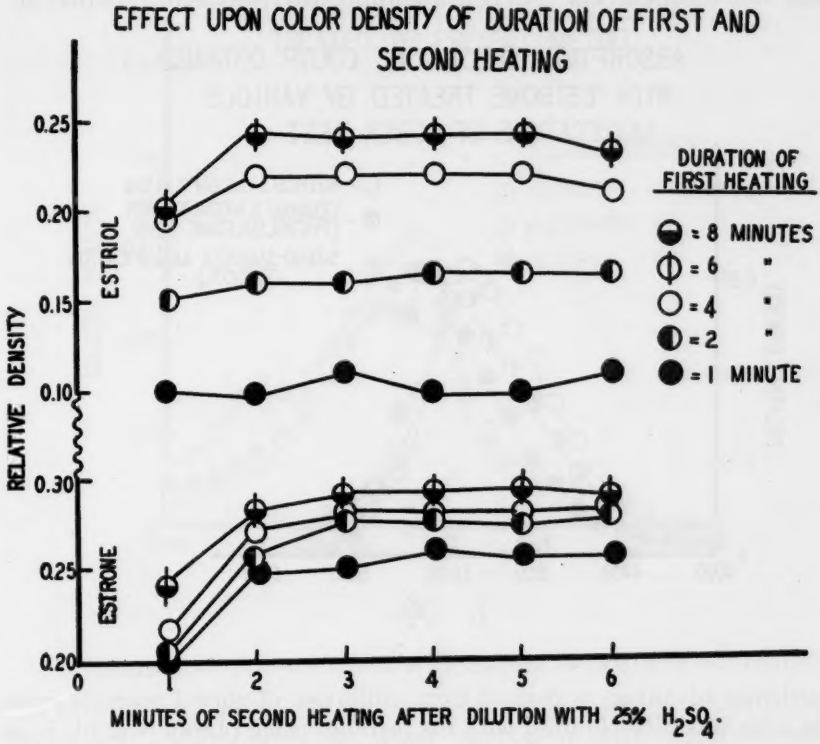

FIG. 2

sulfuric acid and heated at 100° C. for 1, 2, 4, 6, or 8 minutes. All tubes were then diluted with 8 cc. of sulfuric acid (25+75) and heated a second time for the various intervals indicated in Figure 2. Thus for each time of first heating there would be six different times of second heating. The results show that for both estrone and estriol maximum color formation is obtained with a 6 minute first heating, and a 3 minute second heating after dilution. Slightly longer times of first and second heating do not change the maximum values.

3. Absorption Spectra of Color Obtained with Estrone Treated by Various Modifications of the Kober Test:

40 μg. of estrone were treated by various modifications of the Kober test, namely the Szego-Samuels (1943) (8) utilizing guaiacolsulfonic acid, and the Venning et al. (1937) (9) modification using phenolsulfonic acid and the present modification. The absorption spectra of the resulting solutions were obtained (Fig. 3). These absorption curves demonstrate that no

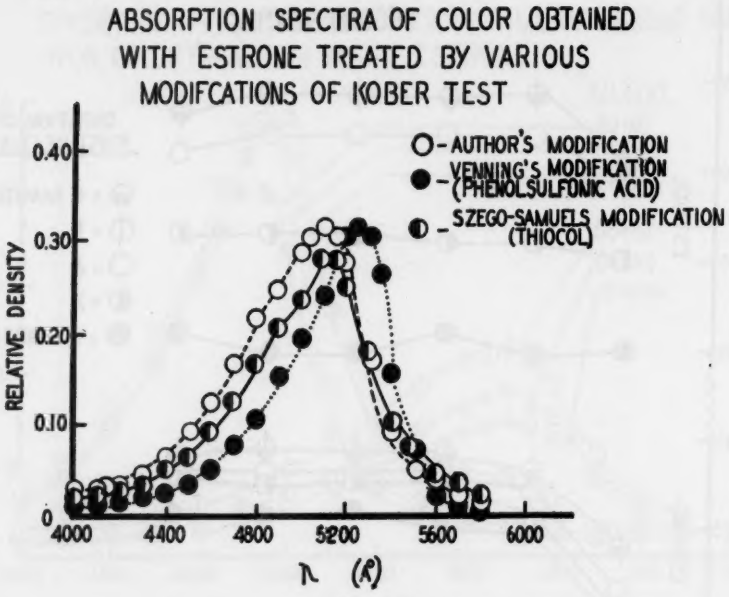

FIG. 3

particular advantage is derived from utilization of phenol reagents since the color intensity obtained with the methods using phenol reagents is of similar magnitude to that obtained with ordinary sulfuric acid. In the case of the phenolsulfonic acid reagent the peak absorption occurs at 5250 Å whereas in the other two cases the peak absorption is at 5100 Å.

There is a distinct visual difference in the solutions because phenol quenches the green fluorescence of the estrogenic steroids. Measurement of the fluorescence of the final solutions obtained by the various methods was carried out with a Klett photofluorimeter. The relative values for fluorescent intensity was 6 units for the method using guaiacolsulfonic acid; 7 units for the phenolsulfonic acid method; and 103 for the present modification. These data demonstrate the quenching effect of the phenol reagents upon the fluorescence.

4. Stability of Color:

A set of tubes containing estrone treated by the present method was kept at room temperature and readings were taken at ½ hour intervals. Up to a period of four hours no change in color density was noted.

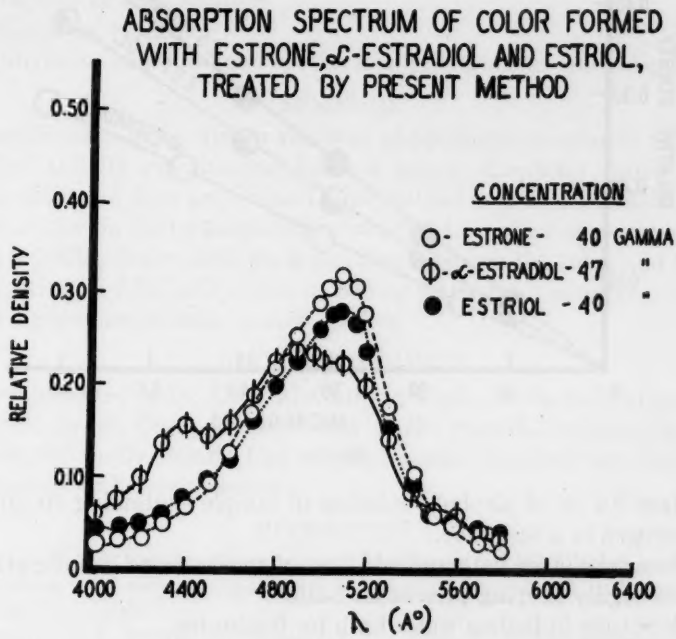

FIG. 4

5. Comparison of Colors Formed With Estrone, Estriol and α-estradiol:

Estrone (40 μg.), estriol (40 μg.) and α-estradiol (47 μg.) were treated by the present modification of the Kober reaction. Absorption curves were obtained (Fig. 4). These curves show that the color formed by estrone and estriol are similar, but differ somewhat in intensity. α-estradiol, however, shows a different absorption spectrum having peaks at 4400 Å, 4900 Å and 5100 Å. However, calibration curves prepared for each of these materials using the absorption at 5100 Å show adherence to Beer's law between the concentration of 5–60 μg. (Fig. 5). The slope of each differs, being 0.725, 0.625 and 0.45 respectively for estrone, estriol and α-estradiol.

6. Description of Method:

Based on the aforegoing observations the following procedure has been devised for the determination of natural estrogens:

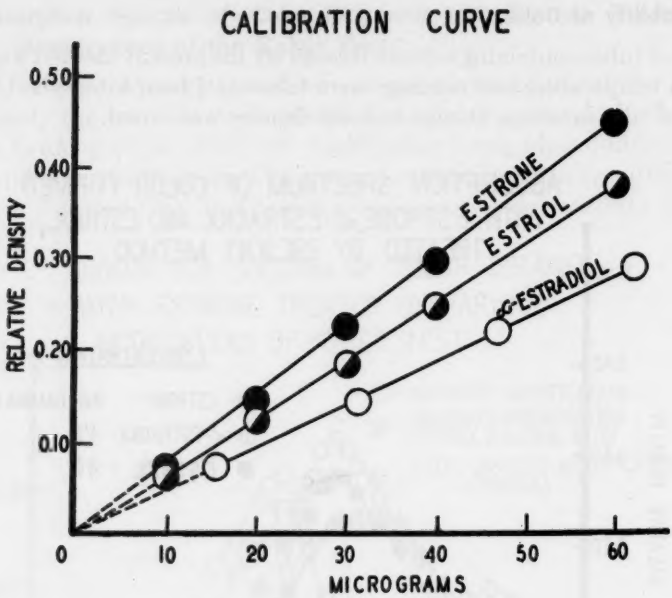

FIG. 5

a) Place 0.4 cc. of alcoholic solution of sample containing 10–50 μg. of estrogen in a test tube.
b) Place tube in ice bath and add 2 cc. of concentrated sulfuric acid. Stir thoroughly keeping tube in ice bath.
c) Place tube in boiling water bath for 6 minutes.
d) Remove tube, add 8 cc. of (25+75) sulfuric acid and reheat in boiling water for three minutes.
e) Cool and read color in photometer using a 5100 Å filter.

DISCUSSION

Kober (1931) (4) in his original paper noted the formation of a red color upon the addition of concentrated sulfuric acid to estrone, heating and diluting with water, but stated that the color formed was too weak for colorimetric measurement. He also noted that an intense greenish fluorescence was present which interfered with colorimetric readings but which was quenched by addition of phenol with an increase in the intensity of the red color. We believe that our data (Fig. 3) show that this increase in intensity is only visually apparent due to quenching of the green fluorescence and that it is not a real increase when measured photometrically.

The temperature of 100°C. for heating was arbitrarily chosen since it is easiest to control. However, lower or higher temperatures would give similar results provided the time of heating was prolonged or shortened corre-

spondingly. This was indicated by the fact that, after the first heating and dilution, similar color intensities were obtained by reheating for 3 minutes at 100° C. or allowing the solution to remain at room temperature for one hour. The advantages of this method are:

1. Reproducibility of the data with different batches of sulfuric acid.
2. Stability of final solution.
3. No special reagent.
4. Anhydrous condition and special manipulations are unnecessary.

SUMMARY

A modification of the Kober reaction which omits the phenol reagent is described and its experimental basis is given. Simplicity, rapidity and reproducibility of data are claimed. The method is as follows: Add 2 cc. of sulfuric acid, c.p., to the sample dissolved in 0.4 cc. of ethanol and heat the tube in a boiling water bath for 6 minutes. Remove the tube, add 8 cc. of sulfuric acid (25+75) and reheat in boiling water for 3 minutes. Cool and read in a photometer using a 5100 Å filter.

ACKNOWLEDGMENTS

We are indebted to Dr. D. A. McGinty of Parke Davis and Company for the estriol, to Dr. Oscar Wintersteiner of the Squibb Institute for the α-estradiol and to Dr. Nettie Coy of the Squibb Chemical and Biological Laboratories for her cooperation.

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EXPERIMENTAL USE OF TESTOSTERONE COMPOUNDS IN PREMATURE INFANTS

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IN A preliminary report Shelton and Varden (11) demonstrated that the oral administration of methyl testosterone to premature infants of both sexes, weighing less than 2000 grams, resulted in an apparent increase of expected survival, with concomitant minimal initial weight loss and early and sustained weight increase throughout the period of treatment.

In an effort to obtain more conclusive data than the initial study provided, testosterone compounds were administered to a much larger group of infants on the premature wards of the Los Angeles County General Hospital, over a period of several months, according to the following procedure:

All premature infants weighing less than 2000 grams were divided into three groups in rotation of entry, with no regard for sex or weight. The first group received methyl testosterone 5 mg. daily in propylene glycol, placed directly on the tongue immediately preceding an oral feeding or gavage. The second group received 4 mg. daily of testosterone propionate intragluteally; while the third group acted as the control. The testosterone was started at the end of the twelfth hour and was continued for three weeks. With the exception of the testosterone, all the infants received the same routine care which is available in any large premature station. A certain number in each group received oxygen or carbogen, if indicated, along with clysis or gavage when necessary. Routine ward orders for care applied to all three groups and the usual vicissitudes associated with premature care seemed to be present in all groups. A chart was devised by which the weight, alertness, type of stool, amount of lanugo, size of the clitoris and penis, and number of erections could be followed on each infant every day. A special registered nurse limited herself to this work, so that observations by the same individual were made from day to day throughout the experiment. For obvious reasons, it was decided that blood and urine studies were not justified in such an experiment, and that the length of time required to regain the birth weight and that required to gain to 2500 grams would be used as the ultimate basis for evaluation of treatment.

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At one interval during the period of observation the premature wards were beset with an epidemic of severe bronchiolitis. Upon review of the statistics, however, it was found that there were as many controls as those treated who had suffered from this disorder, and it was decided to include all of the infants in the final results with no regard for the obvious overall impediment of their weight gain and general development.

Treatment was begun in all infants at the end of 12 hours. Since it was felt, however, that an additional 36 hours were required before an effective level of testosterone could be expected, only those infants surviving 48 hours are included in this study.

Results—During the five-month observation period there were one hundred and twenty-four entries to the premature wards. Of these, twenty were stillborn, twenty-three did not survive beyond the first forty-eight hours; and seven died while on testosterone after the first forty-eight hours. Of these, two were in the control group, three in the testosterone propionate group, and two on methyl testosterone. Post-mortem examinations revealed congenital defects not compatible with life in two, intracranial hemorrhage in one, and non-specific "cardio-respiratory failure of the premature" in the remaining. Although in the original work by Shelton and Varden (11) it was felt by the observer closest to the infants and by the premature nurses that there was objective evidence of greater activity and maturity in the treated infants, no marked difference in behavior between the controls and the treated infants was observed in this series. Because of the epidemic and inadequate help, however, it is possible that the general vigor of the infants was much more difficult to evaluate in this series than in the first group, which were observed by a number of individuals in a private institution.

No untoward effects were observed in either of the two treated groups. It had been speculated by the authors that hoarseness in the cry of the treated females might be one of the signs of an untoward effect. As much cry as is expressed by a premature did not reveal any alteration in pitch in either sex in the treated group. There was no abnormal hair growth and no objective enlargement of the genitalia in either sex.

The seventy-four cases were divided into three groups: twenty in the control group, thirty in the methyl testosterone group, and twenty-four in the testosterone propionate group. These infants were further divided by birth weight into two classifications, 1000 to 1500 grams and those weighing between 1500 and 2000 grams. As 2500 grams is the American Academy of Pediatrics accepted level for normal infants, it was decided to tabulate the days required to regain birth weight, and the days required to regain to a 2500 gram level. The statistics reveal a significant difference between the controls and testosterone-treated infants.

Table 1 shows that the control infants in the 1000 to 1500 gram weight groups required on an average of 14.7 days to regain their birth weight, whereas those on methyl testosterone in the same weight group required only an average of 9.0 days, and those on testosterone propionate 7.5 days. Thus, there was a 50 per cent reduction in the treated over the control group in time required to regain birth weight. In analyzing the 1500 to 2000 gram group, it is shown that the controls required 11.9 days to regain their birth weight, in contrast to 7.8 days for the methyl and 9.8 days for the

TABLE 1

Weight	Treatment	Number of Cases	Days to Regain Birth Weight	Days to Gain to 2500 Gm.
1000 to 1500 Gm.	Control	6	14.7 days	58.8 days
	Methyl Test	6	9.0 days	56.9 days
	Test Prop.	4	7.5 days	55.0 days
1500 to 2000 Gm.	Control	14	11.9 days	42.1 days
	Methyl Test	24	7.8 days	32.6 days
	Test Prop.	20	9.8 days	35.1 days

testosterone propionate infants, which is also an apparently significant trend. In considering total hospital stay, those treated with testosterone compounds again demonstrated a variance from the control groups. The 1000 to 1500 gram control group required 58.8 days to gain to 2500 grams in contrast to 56.9 days for the methyl testosterone group and 55.0 days for those infants on testosterone propionate. The 1500 to 2000 gram controls took 42.1 days to reach 2500 grams, in contrast to 32.6 days for the methyl testosterone infants and 35.1 days for those on testosterone propionate.

An even more significant indication of the benefits obtained from the use of testosterone compounds is shown in Table 2. Here four sets of twins were employed. In three sets the larger of the two (the one with the greater chance for survival) was used as the control, while the smaller was given testosterone. All four of the twins on the testosterone compounds, who, theoretically should have taken longer to regain their birth weight, did so in a much shorter period than their larger, more mature siblings, who were used as controls. In three of the cases the birth weight was maintained from the start, while in the fourth there was an approximate 50 per cent reduction in the anticipated number of days required to regain the birth weight. Again the days required to gain to 2500 grams shows a reduction in the

twin treated with testosterone in three out of the four, while in the fourth set only two days longer were required to bring the smaller infant to maturity.

It is quite likely that the early weight gain or at least the maintenance of birth weight, so commonly observed in these infants, is due to salt and water retention. However no instance of edema was observed. The weight gain up to 2500 Gm. apparently represents a true somatic increment since,

TABLE 2

Twins	Birth Weight	Type Treat.	Days to Regain Birth Weight	Days to Gain to 2500 Gm.
S. #1	1956 Gm.	Control	17 days	32 days
S. #2	1871 Gm.	Test. Prop.	No days—maintained B. wt.	22 days
M. #1	2041 Gm.	Control	9 days	25 days
M. #2	1816 Gm.	Test. Prop.	5 days	17 days
B. #1	1531 Gm.	Control	7 days	50 days
B. #2	1276 Gm.	Methyl. Test.	No days—maintained B. wt.	52 days
P. #1	1446 Gm.	Control	13 days	53 days
P. #2	1531 Gm.	Test. Prop.	No days—maintained B. wt.	41 days

in those infants followed after withdrawal of the hormone no sharp weight loss was seen.

The dose of both methyl testosterone and of the propionate were chosen arbitrarily, and are probably higher than those required to obtain a maximum metabolic effect. In comparing the methyl testosterone with the testosterone propionate, no significant differences could be observed. Testosterone propionate perhaps did offer advantages over oral administration in the accuracy of dosage, but the ease of administration of methyl testosterone in propylene glycol, either on the tongue or in the feeding, and the freedom from the infection hazard is worthy of consideration.

DISCUSSION

It is now generally accepted that testosterone propionate and methyl testosterone cause a decline in urinary nitrogen, reflected in the urea frac-

tion, unaccompanied by an increase in the plasma protein, non-protein nitrogen, urea, or hemoglobin concentrations. As there is no change in the amount of fecal nitrogen, it is evident that this retention of nitrogen must have some tissue building significance. There is also a decline in urinary sodium, associated with a somewhat smaller decline in urinary chloride; a decline in urinary potassium and total urinary volume. The evident end result of this is in water retention and a general protein anabolism, noticeable in the genital tissue, but more objectivized in the soma, with resultant increase in growth and muscle hypertrophy. These effects have been demonstrated by Rubinstein and Solomon in the rat (9); Kochakian and Marlin in the dog (5), (6), (7); Kenyon, et al. in the adult (2), (3), (4); and by Wilkins, Fleischman, and others, in immature children (12). Shay, et al. (10) found that testosterone stimulated growth in normal female rats, but no effect on growth was observed by them when male rats were injected. From this it might be inferred that the male, when surfeited with autogenous hormones, is incapable of further response, but the prematures of both sexes, who are devoid of this physiological activity, might profit by such stimulation at the most crucial period of their existence.

It is believed that with the doses used and especially the sharp period of time during which it was administered, the action of the testosterone should have no effect on osseous development or on epiphyseal closure. McCullagh (8) has shown that small doses of testosterone propionate (15–60 mg. per week) for fifteen months caused no distinct advancement in epiphyseal age, but that doses of 105 mg. or more per week, administered over a period of eight months, did lead to an increase of about two years in epiphyseal development. Gordon and Fields (1) using doses of 20–50 mg. of testosterone propionate per week on pre-puberal and adolescent boys found no instance of premature closure of the epiphysis or of stunting of growth after extended periods of treatment. However ridiculous, some physicians continue to be obsessed with the idea that the administration of testosterone to the infants, for even a few weeks, might interfere with their future growth. For this reason, we have compared roentgenograms of the wrist and knee of the treated individuals, at intervals throughout the first year, with the controls, and, as anticipated, found no difference.

SUMMARY

1) Seventy-four premature infants of both sexes under 2000 grams entering the Los Angeles County General Hospital were divided into three groups: one a control; one placed on methyl testosterone, 5 mg. daily; and one placed on testosterone propionate, 4 mg. daily.

2) A distinct shortening in the time required to regain birth weight and in time required to gain to 2500 grams was noted in both groups receiving testosterone compounds.

3) Four sets of twins were included in the study, and in each instance the one on testosterone showed increased somatic development over its control sibling.

4) It is not our intention to advocate the routine use of testosterone compounds in premature infants, but to suggest such a procedure as an adjunct in the care of a selected group needing metabolic stimulation.

5) No contraindications to the use of testosterone compounds were found.

ACKNOWLEDGMENT

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We are indebted to the Schering Corporation for a grant which enabled us to employ a full time nurse for this study.

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GOITER ON AN IODINE-FREE DIET GROWN BY HYDROPONICS, AND EXCLUDING ANY GOITER NOXA

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VARIOUS workers have experienced difficulty in finding foodstuffs sufficiently low in iodine to produce goiter. This fact has helped to revive the germ ("virus") theory of goiter (1). It seemed of interest to produce goiter in a goiter-free region with precautions against introducing any hypothetical virus. In order to obtain food free from iodine and virus it was grown by hydroponics in a disinfected greenhouse.

According to Frazier (2) goiter is not endemic in this region. Furthermore we did not allow any person or animal with goiter inside the greenhouse. The floor was freshly coated with asphalt and the whole interior, including tanks and window panes, sprayed with 5 per cent DDT and 1 per cent cresol in kerosene. The greenhouse is 25 miles from the nearest city and the water used was rain caught on a glass roof and filtered through amberlite IR4 freshly activated with aluminum ions which would remove any "goiter germ." The reagent chemicals were dissolved in water at pH 10 and filtered through freshly precipitated tri-calcium phosphate which would kill or remove any germs.

Seeds of corn, soybeans and sunflowers were sprouted in broken quartz that had been heated to 150°C in sulfuric acid (a process that would kill any germs). The seedlings were transplanted to holes in the aluminum covers of the tanks. The entering air passed through copper screen with holes 0.6 mm. in diameter, the outgoing air through new galvanized mosquito screen 10 feet from ground and the air to the roots was filtered through 2 inches of packed asbestos followed by carbon filter tubes. From these crops and pure sterile chemicals a diet of 40 per cent sunflower seed, 2.8 per cent soybeans, 40 per cent sucrose, 0.8 per cent NaCl and 16.4 per cent corn oil was prepared. This low percentage of soy beans is not goitrogenic. Six litter mate rats of a "Wistar" colony that had been isolated for 6 years from goitrous animals or humans were put at weaning on this diet. The cages were isolated in a cellophane covered $6 \times 10 \times 10$ foot space. Three of the rats were given water re-distilled from alkali and the other three water containing 10 parts per million of iodine. At the end of 73 days the rats were anesthetized, the thyroids dissected out, placed on small round, pre-

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viously weighed cover glasses and quickly weighed on a chainomatic balance. We had previously found this method as accurate as weighing bottles for two reasons. First, a weighing bottle has a large surface to adsorb moisture; second, during the time it takes for the air in a weighing bottle to come to the temperature of the balance case some of it has been pushed out around the stopper by the water vapor from the fresh tissue.

The rats on the diet and re-distilled water had goiters weighing 41, 42 and 39 mg. per 100 Gm. body weight whereas the rats on the diet and water containing 10 ppm. iodine all had normal thyroids weighing 10 mg. per 100 Gm. body weight. It is thus demonstrated that an iodine-free diet produces goiter.

Greenwald did not present any evidence that goiter is due to a virus and in a second paper (3) borrowed Dieterle and Eugster's term "goiter-noxa" and formulated a number of criteria for iodine workers, one being that the iodine added to prevent goiter not be greater than in ordinary foodstuffs. We have analyzed food pellets used for controls by other workers and found them to contain more iodine than the water we gave our controls.

The goiters produced on the hydroponics diet in a non-goitrous region were twice as large as goiters produced in a goitrous region in rats of goitrous mothers (4).

Conclusion: Goiter was produced by a lack of iodine.

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PHEOCHROMOCYTOMA WITH DIABETES*

A CASE REPORT AND DISCUSSION

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HYPERTENSION and glycosuria are conditions which in the vast majority of cases are not accessible to causal therapy. Yet there is a small—but important—group of patients with high blood pressure, paroxysmal or persistent, and with glycosuria, transitory or even permanent, who can find curative treatment, if the correct diagnosis of their disease is made in time. These are the patients with pheochromocytoma. The pheochromocytoma is an endocrine tumor of adrenal medullary tissue and produces epinephrine. Its active cells are chromaffin and give a brownish stain when exposed to a solution of potassium bichromate; it is from this reaction, that the name pheochromocytoma (brown cell tumor) was derived.

The adrenal medulla originates from the sympathetic nerve system and the pheochromocyte is one of the final developmental forms of the sympathogonia, the stem cell of the sympathetic nerve system. It is therefore easily understandable that only those tumors of the adrenal medulla are hormone-producing in which the differentiation of the stem cells has progressed to the stage of the pheochromocyte. On the other hand, pheochromocytoma may develop outside of the adrenal medulla wherever sympathetic nerve cells are present, as for instance in the sympathetic glands along the aorta and the carotid body. Table 1 shows the pheochromocytoma in its relation to the other non-endocrine tumors of the sympathetic nerve system.

Like most endocrine tumors the pheochromocytoma manifests itself more by symptoms of hormonal overproduction than by invasive growth and metastases, which in fact are rather rare. Its "malignancy" is due mainly to the serious effects of the overflow of its incretoric product upon the general metabolism and particularly the cardio-vascular system. But unlike the cortical tumors of the adrenals or the pancreatic islet cell tumors the symptoms of the pheochromocytoma are rather unspecific and may occur in numerous other conditions of functional or organic origin, from

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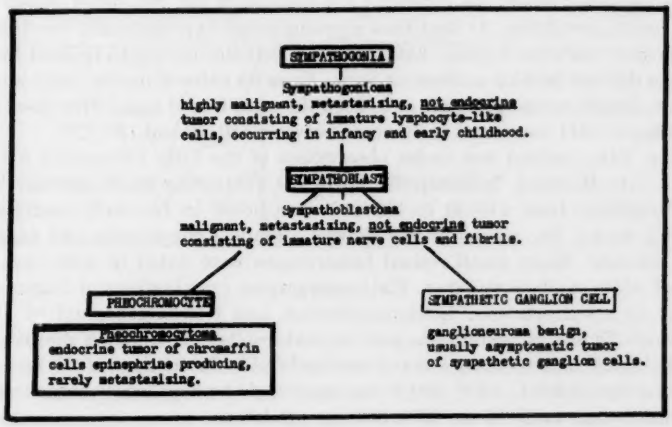
neurocirculatory asthenia to malignant hypertension. Therefore its clinical diagnosis is rather difficult and is made more frequently where the physician is aware of its occurrence and considers the possibility of its presence in a given case, than when a correct diagnosis is expected to be the result of a series of objective laboratory tests. The pheochromocytoma is a rare disease, but the number of clinically diagnosed and surgically cured cases has been increasing rapidly since the first description of the syndrome, and this increase is certainly not due to an increased incidence of the disease, but only to the better diagnosis.

It was more than 25 years after the discovery of the medullary hormone of the adrenals (1895) that the clinical syndrome of the medullary tumor

TABLE 1



was described by Labbé, Tinel and Doumer in 1922 (9). Humphreys (8) was able to collect 103 cases from the literature up till 1939, but most of these were postmortem observations. In 1937 Howard and Barker (7) reported 18 clinically diagnosed cases, in 1941 Biskind and co-workers (1) analyzed 33 clinically diagnosed and operated cases from the world literature, out of which 26 had been cured surgically, and in 1944 Cahill (4) reported 9 cases of his own observation. In Billings Hospital 5 cases were seen in recent years, 3 of them reported by Brunschwig, Humphreys and co-workers (2). During the 2 years 1945–1946 at least 24 individual reports of successful removal of pheochromocytoma have appeared in the world literature.

It is gratifying to note the increasing frequency with which diagnosis is made or suggested by the practicing physician out in the field instead of being left to the rear echelon of the well equipped diagnostic hospital. The

credit for the correct differential diagnosis of the case, here to be discussed, goes to the family physician who referred the patient to the hospital for further observation and treatment.

This patient's case is of special interest because among its presenting signs and symptoms were not only glycosuria but a typical diabetes. As far as we have been able to ascertain, this seems to be the second case of its kind to be reported, the first case being described by Duncan, Semans and Howard in 1944 (6).

The patient, *M.L.E.* (*#228050*), was a 20 yr. old white female who was admitted to the Albert Merritt Billings Hospital of the University of Chicago on 7-11-44. She had been in excellent health until about 1942 when she first noticed periodically recurrent headaches, increasing nervousness, occasional attacks of mild dizziness and hyperhidrosis, nocturia and polyuria. It was not until two years later that she was seen by a physician whom she consulted not because of her symptoms but in order to obtain a health certificate. At that time glycosuria and hyperglycemia were found and the diagnosis of diabetes mellitus was made. Her diabetes proved to be hard to control, though she did not develop acidosis or coma. Even 85 units of insulin daily were insufficient to maintain normo-glycemia or to keep the urine free of sugar. Her blood pressure in the spring of 1944 was found to fluctuate between 110/70 and 180/120.

In May, 1944, patient was under observation of the Lilly Laboratory for Clinical Research, City Hospital, Indianapolis. There the fluctuating blood pressure was confirmed. Variations from 110/50 to 170/110 were noted in two daily readings over a period of 3 weeks. The retinal vessels showed a grade 1 constriction and questionable grade 1 sclerosis. Many small retinal hemorrhages were noted in either eye and an occasional white exudate was seen. Plethysmographic examinations of fingers and toes indicated no arteriosclerosis. Electrocardiogram and teleröntgenogram of the chest were normal. Kidney function tests gave normal results. The diabetes showed again an unusually high insulin requirement and unusually wide fluctuations of the fasting blood sugar. On a diet of 200 C, 86 P, 100 F, the urine could be kept nearly sugar free but the fasting blood sugar ranged from 72 to 367 mg. per 100 cc.

On admission to Billings Hospital, the patient appeared apprehensive and complained about nervousness, headaches and excessive perspiration. Family history and history of previous illnesses were not contributory. Menstrual history was normal. The physical examination revealed a well developed female with a slight excess of dark hair on face, breast, abdomen and thighs. The patient was not certain whether or not her hair growth had increased recently. Her weight was 48.7 kg. (107 lb.), height 155 cm. (5 ft. 1 in.). The skin was moist and there was mild tremor manum and moderate hyperreflexia. The eye reflexes were normal. No exophthalmos or lid lag was noted. The thyroid was not enlarged. The chest was symmetrical and normal to auscultation and percussion. The heart appeared to be of normal size, rate and rhythm. There were no murmurs. The blood pressure was 170/120; the abdomen was soft, liver and spleen did not appear enlarged, no masses were palpated in the abdomen. The pelvic examination was normal. Fundoscopic examination (Dr. Spiro) revealed many hemorrhages of various sizes over both retinae, surrounded by irregular white exudates. The retinae appeared edematous, the veins were markedly engorged and tortuous, the arteries only slightly constricted. The crossing changes were marked.

The blood count was normal, the serology negative, the urinalysis negative for albumin but positive for sugar, the specific gravity 1.027, the sediment normal. X-ray

examinations of chest and skull were normal, intravenous and retrograde pyelograms showed no abnormalities in kidney shape or function; a small bony defect, in the right ilium near the sacro-iliac joint was observed. (This unidentified lesion was reexamined at intervals during the subsequent two years and has not changed in size.) The electrocardiogram was again normal.

Blood pressure readings were taken 4 times daily and again marked daily fluctuations were noted. Fig. 1 demonstrates the course of the blood pressure. Sodium amytal and cold pressure tests gave normal responses. The kidney function was studied extensively with urea-, inulin- and diodrast-clearances, and was found to be normal.

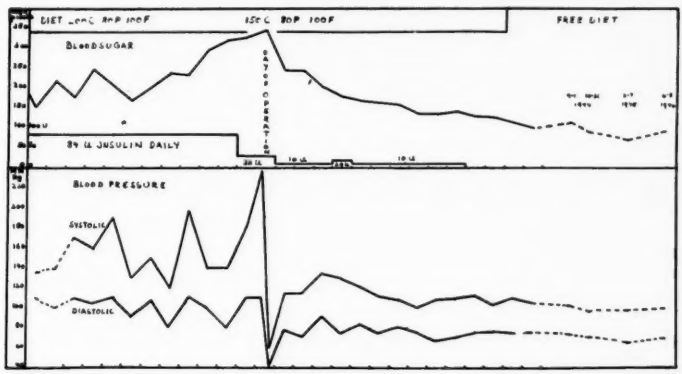


FIG. 1

The diabetes remained unstable, with fluctuations of the fasting blood sugar between 81 and 290 on a stable diet (200–80–100) and 85 units of insulin. The course of the blood sugar and the insulin dosage are demonstrated on Fig. 1. An insulin tolerance test showed an unusual response with a slight drop of the blood sugar during the first 15 minutes (from 225 to 190) and with a rapid return to a value higher than the fasting level (275) within 2 hours. Such a curve may be obtained if adrenaline is given together with or shortly after insulin. Because of the rather mild hirsutism the question of an adrenal cortical tumor was discussed. The 17-ketosteroid excretion was determined and found to be normal. The blood electrocytes were Cl 96 *m*Eq./L, Na 136.8 *m*Eq./L and K 4.5 *m*Eq./L.

It was felt that pheochromocytoma was the most likely possibility in the differential diagnosis and surgical exploration was recommended. This was performed by Dr. William E. Adams on 7-24. The abdominal approach was used. Exploration of the region of the right adrenal gland revealed a mass approximately 5 × 3 cm. in size which was medial and superior to the kidney and in the medial aspect of the adrenal gland. The left adrenal appeared normal. The tumor mass was freed from the surrounding tissue, the blood vessels of the tumor were clamped, and the tumor was then removed. During this procedure the blood pressure rose to 240/120 and then fell to a shock level of 60/40; under ephedrine the blood pressure returned to normal levels within a short period of time.

Morphological-pathologic examination of the tumor by Dr. E. Humphreys revealed the following: The tumor, 3 × 5 cm. in diameter, consisted of rather homogenous tissue

and had a fibrous capsule. Two pieces of the tumor were immersed in 3 per cent bichromate solution and developed the brownish color typical of pheochromocytoma. Microscopically the tumor was composed of cells arranged in nests and cords separated by small blood vessels. In many regions, the appearance was identical with that of the normal adrenal medulla.

Post-operative course: The patient had an uneventful recovery from her operation. The blood pressure remained within normal limits throughout and was 120/90 on 5-6-46, about two years after the operation (Fig. 1). Headaches, dizziness, nervousness and hyperhidrosis have disappeared completely and permanently.

Reexamination of the fundi showed gradual disappearance of the bilateral retinopathy. By May 1946 the vessels of the fundi had returned to normal appearance; neither hemorrhages, nor exudates or retinal edema were visualized.

TABLE 2

THE POST OPERATIVE IMPROVEMENT OF THE ORAL GLUCOSE TOLERANCE IN A CASE OF PHEOCHROMOCYTOMA WITH DIABETES

Date	1944 8-9		1945 2-7		1946 6-5	
Time	Blood sugar	Glycosuria	Blood sugar	Glycosuria	Blood sugar	Glycosuria
Fasting	144	0	71	0	91	0
30'	258	0	169	0	192	0
120'	282	++	209	+	172	0
180'	242	++	190	+	124	0

The diabetes showed the most spectacular change. Immediately after the operation the fasting blood sugar level began to drop, in spite of the fact that the daily insulin dose had been decreased. Within 10 days the blood sugar had returned to normal and the urine remained sugar free. Only 10 units insulin daily were given during that period. The patient then was permitted a free diet and insulin was discontinued. Her fasting blood sugar remained normal and her urine sugar-free. To check this apparent cure of her diabetes an oral glucose tolerance test was performed the day prior to her discharge from the hospital. This test showed a typical diabetic curve and thus revealed that though the diabetes had improved markedly, it had not been cured (Table 2). After discharge from the hospital the fasting blood sugar was found to be normal at various examinations and the urine to be sugar-free at all times. Annual glucose tolerance tests however showed the persistence of a mild diabetic state.

COMMENT

Almost all of the typical features of a pheochromocytoma are illustrated by this patient's history: The fluctuant hypertension, the palpitations, dizziness and headaches are classical symptoms of epinephrine overactivity. Hyperhidrosis, nervousness and tremor manum are encountered frequently as associated symptoms. These, if very marked and accompanied by an elevated basal metabolic rate, may occasionally mislead to the differential diagnosis of hyperthyroidism. It should be remembered

that epinephrine itself may cause hypermetabolism. The age of the patient and the location of the tumor illustrate the fact that pheochromocytomata occur most commonly in young or middle aged persons and are more frequently found in the right than in the left side of the abdomen. The occurrence of the relatively minor fluctuations of the blood pressure during the operation reminds of the sometimes very serious complications of surgery. Manipulation of the tumor may express large amounts of adrenaline into the circulation which may suddenly precipitate an extremely high rise of blood pressure, peripheral vasoconstriction, lung edema and death. Ligation of all blood supply prior to removal of the tumor, as was done in this case, prevents this hazard. On the other hand, circulatory shock threatens after extirpation of the tumor and substitution therapy with epinephrine and cortical extract is frequently necessary for a short period postoperatively. Because of the occasional multiplicity of pheochromocytomata and the importance of examining both adrenals prior to extirpation of the diseased one, the abdominal approach, as used here, has been accepted generally as superior to the retroperitoneal dorsal method, which permits only unilateral inspection. The return of the blood pressure to a normal level and the practically complete disappearance of the retinal changes are the gratifying and common results of surgery if done in time.

It is noteworthy that, except for the diabetes, the symptoms in our patient were rather mild. None of them alone would have been indicative of a pheochromocytoma, yet the combination of all was very suggestive of the diagnosis which proved to be correct at surgery. This supports Howard's dictum that "when diabetes mellitus, hypertension aand hypermetabolism are encountered, the possibility of pheochromocytoma should be considered," to which we may add that this consideration is warranted especially if the patient is of middle age or younger and if no evidence of arteriosclerosis or primary kidney disease is found. Occasionally palpation, more frequently a pyelogram or radiography, in combination with perirenal air insufflation will establish the diagnosis, but not infrequently the tumor is so small that nothing short of exploratory laparotomy will prove the presence or absence of a pheochromocytoma. This, of course, should be done only if the symptoms are suggestive, but then it is indicated, indeed. In many instances exploratory laparotomy may mean the difference between a chance for complete cure on one hand and the poor prognosis of progressive hypertension and arteriosclerosis on the other hand, with the added danger of sudden death in a hypertensive crisis.

The course and the significance of the diabetes in our patient deserves a special discussion. There are a few case reports in the literature where a typical diabetic syndrome, persistent hyperglycemia and glycosuria, was associated with pheochromocytoma, but with the exception of the case of

Duncan, Semans and Howard (6), either the diagnosis of pheochromocytoma was made only postmortem or the patient died soon after operation so that no observations about the later course of the diabetes could be made nor could any conclusion be drawn as to the relationship of the diseases. In the only case similar to ours, an insulin resistant diabetes of more than three years' duration improved to such a degree that it could be considered cured for all practical purposes. The patient did not require insulin any longer and was sugar free and normoglycemic on a daily intake of 300 Gm. carbohydrate. Here too, however, decreased glucose tolerance tests were obtained months after removal of the tumor, but the authors were inclined to minimize the significance of this "slight residual defect in the mechanism of carbohydrate disposal." In this as well as in our case the marked, rapid and persistent post-operative improvement of the disturbed carbohydrate metabolism argued strongly against the possibility that the diabetes was a coincidental and independent disease. The relatively high insulin requirement in both instances points in the direction that extra pancreatic factors were at least contributory to the occurrence of hyperglycemia and glycosuria. Overactivity of epinephrine, causing increased glycogenolysis in the liver, was most likely the initial step in this mechanism. As long as the hormone secreting tumor was present the hyperglycemia persisted. After the operation a return to normal, a restitutio ad integrum, seemed to have occurred, but a persistent though latent disturbance of the carbohydrate metabolism became evident under the stress of the glucose tolerance test. It seems to us that this remaining decreased carbohydrate tolerance deserves the same emphasis as is given to the marked clinical improvement. It has long been argued that prolonged hyperglycemia of non-pancreatic origin may lead to irreversible pancreatic islet cell damage and diabetes. The present stand on this problem and the evidence in favor of this hypothesis has been summarized ably by Ricketts (10), and only recently Dohan and Lukens (5) have demonstrated islet cell degeneration in cats, the blood sugar of which had been elevated for prolonged periods of time by glucose injection. The assumption, therefore, seems permissible that the prolonged hyperglycemia which was produced by the pheochromocytoma had exerted irreversible damage upon the blood sugar regulatory mechanism so that a decreased carbohydrate tolerance persisted after removal of the tumor. Unfortunately no biopsies from the pancreas were obtained in our patient. Further clinical observations will have to prove whether such damage is manifested by histological changes in the pancreas.

SUMMARY

A new case of pheochromocytoma with diabetes is reported and the symptomatology of this syndrome is discussed. The persistence of a de-

creased carbohydrate tolerance after successful removal of the tumor seems to give support to the theory that hyperglycemia of extrapancreatic origin may cause irreversible pancreatic islet cell damage and diabetes.

ACKNOWLEDGMENT

The author wishes to acknowledge his indebtedness to Dr. D. B. Marcus of Detroit and Drs. Bruce Peck, R. D. Taylor and A. C. Corcoran of the Lilly Laboratory for Clinical Research, City Hospital, Indianapolis, who supplied pertinent data of the case history and laboratory tests. Grateful acknowledgement is due also to the colleagues at the University of Chicago, Dr. Wm. E. Adams who performed the operation, Dr. E. Humphreys, who reviewed the specimen and to Drs. Milton Landowne and Barbara Spiro for special tests.

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ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later. The Council will meet at 2 P. M. Thursday, June 17th.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

Announcement of Awards and Fellowship of the Association

Nominations for Awards

Three awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma. All nominations, accompanied by a statement of the importance of the nominee's contributions to endocrinology and a bibliography of his most important papers with reprints if possible, should be sent to Dr.T urner's office not later than March 15, 1948.

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of $1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. Edward A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori. No award was made in 1943. No age or special limitation is stipulated by the donor of the award.

THE CIBA AWARD

The Ciba Award, established in 1942, is given in recognition of the meritorious accomplishment of an investigator, not over 35 years of age, in the field of clinical or pre-clinical endocrinology. In 1944 the Award was given to Dr. E. B. Astwood; in 1945 to Dr. Jane Anne Russell; in 1946 to Dr. Martin M. Hoffman and in 1947 to Dr. Choh Hao Li. The Award is for $1,200.00. If within two years of the date of the Award, the recipient chooses to use it to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to $1,800.00.

THE AYERST, McKENNA & HARRISON FELLOWSHIP

The first award of the Ayerst, McKenna & Harrison Fellowship was given to Dr. Samuel Dvoskin in 1947. The fellowship was founded in order to encourage investigation in the field of endocrinology rather than as an award

for work done. The amount of the fellowship is $2,500.00 annually. The nominee must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed the requirements for their Ph. D. or M. D. degree. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals; submit a program of proposed study; indicate one or more institutious where the proposed program will be carried out; submit statement of approval from the investigators with whom he proposes to conduct his research; serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AMERICAN ASSOCIATION FOR THE STUDY OF GOITER

VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. Davison,
Corresponding Secretary

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor; D. A. McGINTY, *Collaborators;* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

PANCREAS

HERZSTEIN, J. AND H. DOLGER. The fetal mortality in women during the prediabetic period. *Am. J. Obst. and Gynec. 51* (3): 420–422 (1946).

Direct interview of 200 married diabetic mothers was carried out. A total of 626 children were born, including 21 stillbirths and 17 neonatal deaths, which yielded a total fatality rate of 6.1 per cent. An analysis of the statistics for 5 year periods showed no marked variations from this figure except in the group who delivered 1 to 5 years before the onset of their diabetes. In this group, there was a fetal mortality of 15.4 per cent. The authors found no greater incidence of stillbirth or neonatal fatality in those who later required insulin than in those who had milder diabetes. They conclude that the fetal mortality during the prediabetic period is no higher than in nondiabetics.—*C.D.D.*

JACKSON, R. L., AND H. G. KELLY. Growth of children with diabetes mellitus in relationship to level of control of the disease. *J. Pediat. 29:* 316 (1946).

The rate of growth of 120 diabetic children has been correlated with the level of control of the diabetes mellitus. It was shown that a much stronger tendency exists for these children to be normal or accelerated than to be retarded. The Iowa City growth charts were used as standards of comparison. In the group before the age of 10 years, the children with excellent diabetic control had a better chance to accelerate than those under lower levels of diabetic control. Those under poor diabetic control, or fair diabetic control did not accelerate. Six of the seven children who grew less than expected, were classified as being either under poor diabetic control or fair diabetic control. Normal growth was found for approximately $\frac{2}{3}$ of those under "fair" and "fair-to-good" diabetic control, but since many of these children had some retardation, compensatory growth might have been effected by a better level of control of the disease. Erratic growth was more frequent for children under fluctuating levels of control. In the group of children after 10 years of age, the fluctuations were more unpredictable and more difficult to evaluate in terms of growth. There was a consistent tendency for growth to be normal or accelerated for those with better levels of control, and to be normal or less than expected for those with "fair" or "poor" diabetic control. In this group were 11 children who were admitted to the clinic after intervals of 2–9 years of poor diabetic control following the onset of the disease. Four of these patients accelerated after coming under

"fair" diabetic control. There was a tendency for girls in the higher levels of diabetic control to mature normally, and for those in the lower levels to be delayed in maturing. A longer-than-average growing period was generally co-existent with delayed menarche. When the onset of diabetes occurred during the years 10–14, it was likely to interfere with the maturation processes. Only $\frac{1}{3}$ of the girls under good-to-excellent diabetic control had delayed menarche, while $\frac{3}{4}$ of the girls under fair-to-poor diabetic control had delayed menarche. The severity of the disease added to the problem of control, since the children with less severe disease tended to maintain higher levels of control. However, the severity did not appear to be the determining factor when the disease was well controlled. The authors emphasize the importance of early and complete management in order to prevent retardation of growth and development. For the children already stunted from improper care, complete management is essential to ameliorate the retardation of growth and development.—*E.C.R. Jr.*,

MILLER, H. C. The effect of diabetic and prediabetic pregnancies on the fetus and newborn infant. *J. Pediat. 29:* 455 (1946).

It has long been recognized that the diabetic state in the mother has often been associated with many unusual phenomena in the fetus and new-born infant. Among the outstanding peculiarities of these infants, there has been noted a high mortality rate, a tendency toward an excessive birth weight and occasional anatomic changes in some of the viscera and glands of internal secretion. Until recently, it has been thought that these peculiarities of the offspring were in large part related to the maternal hyperglycemia and the manner in which it was regulated. Excessive size of the fetus was attributed directly to the fact that the fetus was maintained during pregnancy on a high carbohydrate intake. High fetal mortality was explained either by the occurrence of maternal hyperglycemia or hyperglycemia associated with an inadequate regulation of the diabetes; the high neo-natal mortality was considered to be the result of the hypoglycemia induced in the newborn infant by the overactivity of its pancreatic islet tissue. The basis for this theory rested on the observation that in some infants born to diabetic mothers an actual hypertrophy of the islet tissue could be observed. Recent studies indicate that these previously held theories are improbable. There is considerable evidence to show that all the phenomena observed in infants born to diabetic mothers are to be found among infants born before the onset of maternal diabetes. In this paper, the author reviews this newer evidence and gives statistical data concerning the incidence of fetal and neo-natal mortality, the birth weight, and the occurrence of anatomic changes in the viscera and glands of internal secretion in a series of infants born to diabetic mothers, or to mothers who subsequently became diabetic. The question is raised as to whether the increase in birth weight and the changes in the organs of these infants may be due to an increased amount of pituitary growth hormone which is not only diabetogenic for the mother but also growth-promoting for the fetus. Furthermore, the question is raised as to whether there may be a deficiency in the production of steroid hormones by the placenta during diabetes which is responsible for fetal death and for some of these alterations in the offspring of diabetic or prediabetic mothers.—*E.C.R., Jr.*

ERRATA

After carefully reviewing the article on "Relation of Urinary Steroids to the Diagnosis of Adrenal Cortical Tumors and Adrenal Cortical Hyperplasia: Quantitative and Isolation Studies" by Drs. Edwin J. Kepler and Harold L. Mason which appeared in the Journal of Clinical Endocrinology 7 (8):543-558 (August, 1947), Doctor Harold L. Mason has found the following errors in Table 3, page 551:

1. In case 3, line 1, column 7, the weight of the nonketonic fraction should be 640 mg. instead of 6400, and the value in parenthesis should be 45.7

2. In case 6, line 3, column 8, the weight of dehydroisoandrosterone is given as 1,525 mg. which is more than the total weight of the neutral extract. It should have been explained that this amount of dehydroisoandrosterone included the dehydroisoandrosterone isolated as the sulfate, as well as that which was obtained from the neutral extract after hydrolysis of the urine.